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MICROBIOLOGY OF SEWAGE SLUDGE DISPOSAL IN SOIL

Robert H. Miller

Ohio Agricultural Research and Development Center

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The National Environmental Research Centers provide this multidisciplinary focus through programs engaged in:

- studies on the effects of environmental contaminants on man and the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

The research reported here was conducted for the Ultimate Disposal Section of the Advanced Waste Treatment Research Laboratory to determine soil and climatic factors that affect biological decomposition of digested sewage sludge in soils. Better selection of sludge spreading sites and management of sludge amended soils should result from the use of the information contained in the report.

A. W. Breidenbach, Ph.D.
Director
National Environmental
Research Center, Cincinnati

ABSTRACT

Laboratory studies were conducted to evaluate some of the factors which influence the microbial degradation of anerobically digested sewage sludge in soils and the population of microorganisms involved in these processes.

Anaerobically digested sewage sludge was rather resistant to decomposition with a maximum of about 20% of the sludge carbon evolved as CO_2 in 6 months. The rate of decomposition at the high loading rates of 90 and 224 metric tons/ha of dry solids was found to be independent of differences in soil chemical properties. Differences in soil texture influenced sludge decomposition indirectly by influencing soil aeration under saturated moisture conditions. A relationship was shown between the percent sludge carbon evolved as CO_2 and Monthly Degree Days which will provide a method for predicting the amount of sludge decomposition in a given climatic area based on available temperature data.

Accumulation of soluble soil nitrogen and soluble salts in sludge amended soils could limit the rate of application of sewage sludge to soils. Analyses of the soil solution coupled with plant analyses of Kentucky 31 Fescue have shown that the solubility and plant uptake of some metal ions is increased appreciably, primarily by the lowering of pH associated with nitrification of sludge nitrogen.

Both fungi and bacteria increased in number in response to sludge amendments. Detailed characterization of bacterial isolates showed definite changes in the morphology and physiology of bacteria from sludge amended soils.

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CONTENTS

Page

Abstract iv

List of Figures vi

List of Tables x

Acknowledgements xiii

Sections

I Conclusions 1

II Recommendations 2

III Introduction 3

IV Materials and Methods 5

V Results and Discussion 17

- Phase 1 Measurement of CO_2 Evolution 17
- Phase 2 Microbiological Study 32
- Phase 3 Analyses of the Displaced Soil Solutions 63
- Phase 4 Effects of Anaerobically Digested Sewage Sludge on Kentucky 31 Fescue 98

VI References 115

VII Appendix A 118

FIGURES

No.		Page
1	Assembled CO_2 train with manifold, soil columns, and bubble towers	12
2	Cumulative plot of CO_2-C evolution from sludge amended soils during temperatures equivalent to autumn-winter (Columbus, Ohio)	18
3	Cumulative plot of CO_2-C evolution from sludge amended soils during temperatures equivalent to winter-spring (Columbus, Ohio)	19
4	Cumulative plot of CO_2-C evolution from sludge amended soils during temperatures equivalent to spring-summer (Columbus, Ohio)	21
5	Cumulative plot of CO_2-C evolution from sludge amended soils during temperatues equivalent to summer-autumn (Columbus, Ohio)	22
6	Relationship between % sludge carbon evolved as CO_2 and degree days (90 metric ton/ha amendment)	23
7	Relationship between % sludge carbon evolved as CO_2 and degree days (224 metric ton/ha amendment)	24
8	Cumulative plot of CO_2-C evolution from sludge amended Ottokee sand (autumn-winter temperatures, Columbus, Ohio)	26
9	Cumulative plot of CO_2-C evolution from sludge amended Celina silt loam (autumn-winter temperatures, Columbus, Ohio)	27
10	Cumulative plot of CO_2-C evolution from sludge amended Paulding clay (autumn-winter temperatures, Columbus, Ohio	28
11	Dilution plate counts of bacteria and actinomycetes in sludge amended Ottokee sand as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV)	33
12	Dilution plate counts of bacteria and actinomycetes in sludge amended Celina silt loam as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiment I-IV)	34

No. Page

13 Dilution plate counts of bacteria and actinomycetes in sludge amended Paulding clay as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV) 35

14 Dilution plate counts of anaerobic bacteria in sludge amended Ottokee sand as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV) 37

15 Dilution plate counts of anaerobic bacteria in sludge amended Celina silt loam as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV) 38

16 Dilution plate counts of anaerobic bacteria in sludge amended Paulding clay as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV) 39

17 Dilution plate counts of soil fungi in sludge amended Ottokee sand as influenced by loading rate, soil moisture content, time of incubation, and temperature of incubation (Experiments I-IV) 40

18 Dilution plate counts of soil fungi in sludge amended Celina silt loam as influenced by loading rate, soil moisture content, time of incubation, and temperature of incubation (Experiments I-IV) 41

19 Dilution plate counts of soil fungi in sludge amended Paulding clay as influenced by loading rate, soil moisture content, time of incubation, and temperature of incubation (Experiments I-IV) 42

20 Soil pH in incubated columns of sludge amended and control Ottokee sand (Experiments I-IV) 57

21 Soil pH in incubated columns of sludge amended and control Celina silt loam (Experiments I-IV) 58

22 Soil pH in incubated columns of sludge amended and control Paulding clay (Experiments I-IV) 59

23 Relationship of solution pH to soil pH (Ottokee sand) 60

24 Relationship of solution pH to soil pH (Celina silt loam) 61

No.		Page
25	Relationship of solution pH to soil pH (Paulding clay)	62
26	Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated at field capacity (Experiment I, autumn-winter)	71
27	Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated under saturated conditions (Experiment I, autumn-winter)	72
28	Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated at field capacity(Experiment II, winter-spring)	73
29	Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated under saturated conditions (Experiment II, winter-spring)	74
30	Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated at field capacity (Experiment III, spring-summer)	75
31	Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated under saturated conditions (Experiment III, spring-summer)	76
32	Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated at field capacity (Experiment IV, summer-autumn)	77
33	Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated under saturated conditions (Experiment IV, summer-autumn)	78
34	Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated at field capacity (Experiment I, autumn-winter)	79
35	Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated under saturated conditions (Experiment I. autumn-winter)	80
36	Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated at field capacity (Experiment II, winter-spring)	81
37	Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated under saturated conditions (Experiment II, winter-spring)	82
38	Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated at field capacity (Experiment III, spring-summer)	83

No.		Page
39	Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated under saturated conditions (Experiment III, spring-summer)	84
40	Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated at field capacity (Experiment IV, summer-autumn)	85
41	Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated under saturated conditions (Experiment IV, summer-autumn)	86
42	Soluble ammonium and nitrate nitrogen in displaced soil solutions from sludge amended and control Paulding clay (Experiment I, autumn-winter)	87
43	Soluble ammonium and nitrate nitrogen in displaced soil solutions from sludge amended and control Paulding clay (Experiment II, winter-spring)	88
44	Phosphorus content of Kentucky 31 Fescue grown in sludge amended soils	104
45	Potassium content of Kentucky 31 Fescue grown in sludge amended soils	106
46	Calcium in content of Kentucky 31 Fescue grown in sludge amended soils	107
47	Magnesium content of Kentucky 31 Fescue grown in sludge amended soils	108
48	Sodium content of Kentucky Fescue grown in sludge amended soils	109
49	Zinc content of Kentucky 31 Fescue grown in sludge amended soils	110
50	Copper content of Kentucky 31 Fescue grown in sludge amended soils	111
51	Boron content of Kentucky 31 Fescue grown in sludge amended soils	112
52	Manganese content of Kentucky 31 Fescue grown in sludge amended soils	114

TABLES

No.		Page
1	Research plan: Microbiology of sewage sludge decomposition in soils	6
2	Temperature program for sludge disposal study	7
3	Physical and chemical properties of experimental soils	8
4	Analyses of digested sewage sludge from Columbus Jackson Pike Plant (three sampling times)	9
5	Analyses of freeze dried anaerobically digested sewage sludge	10
6	Characters for which all bacteria were examined	14
7	Sludge decomposition in Ottokee sand as affected by seasonal temperatures	29
8	Sludge decomposition in Celina silt loam as affected by seasonal temperatures	30
9	Sludge decomposition in Paulding clay as affected by seasonal temperatures	31
10	Relative survival of indicator bacteria in soils amended with anaerobically digested sewage sludge	44
11	Distribution of bacterial isolates obtained from dilution plating	46
12	Morphological and cultural characteristics of bacterial isolates from the Ottokee sand and Celina silt loam soils (Experiments I & II)	48
13	Morphological characteristics of bacterial isolates from the Ottokee sand and Celina silt loam soils (Experiments III & IV)	49
14	Morphological characteristics of bacterial isolates from Paulding clay soils (Experiments I-IV)	50
15	Growth characteristics of bacterial isolates	51
16	Biochemical and enzymatic activity of bacterial isolates	52
17	Acid production from selected carbohydrates (aerobic)	53

No.		Page
18	Acid production from selected carbohydrates (anaerobic)	54
19	Sensitivity of bacterial isolates to seven selected antibiotics	55
20	Specific conductance of soil solutions displaced from sludge amended Ottokee sand	65
21	Specific conductance of soil solutions displaced from sludge amended Celina silt loam	66
22	Specific conductance of soil solutions displaced from sludge amended Paulding clay	67
23	Relationship of crop response to soil salinity expressed in terms of the conductivity of the saturation extract (Richards, 1954)	64
24	Organic matter contents of soil solutions displaced from sludge amended soils	69
25	Percentage of the organic nitrogen of anaerobically digested sewage sludge appearing in the soil solution after 6 months incubation	90
26	Concentration of Ca in displaced soils solutions from sludge amended soils	92
27	Concentration of Mg in displaced soil solutions from sludge amended soils	93
28	Concentration of Na in displaced soil solutions from sludge amended soils	94
29	Concentration of Zn in displaced soil solutions from sludge amended soils	95
30	Concentration of Cu in displaced soil solutions from sludge amended soils	96
31	Concentration of Mn in displaced soil solutions from sludge amended soils	97
32	Effect of anaerobically digested sewage sludge on germination of Kentucky 31 Fescue in sludge amended soils	99
33	Effect of anaerobically digested sewage sludge on dry matter yield of Kentucky 31 Fescue. Data expressed as ratio, Sludge treatment/Control	101

No.		Page
34	Effect of anaerobically digested sewage sludge on dry matter yield of Kentucky 31 Fescue. Data expressed as the mean of dry weight (g) for the 1, 3 and 6 months incubation	102
35	Nitrogen and sulfur content of Kentucky 31 Fescue grown in sludge amended soils	103

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CONCLUSIONS

1. The organic compounds in anaerobically digested sewage sludge are rather resistant to further microbial degradation in soil. Thus land disposal of sewage sludge would be expected to increase the organic matter content of the soil and markedly change its physical and chemical properties.

2. The rate of sewage sludge decomposition at the high loading rates used in this study (90 & 224 metric tons/ha) is largely independent of soil chemical properties. Differences in soil pH, initial fertility, organic matter content, nitrogen content, etc. would not influence the degradative activity of the microbial population.

3. Soil texture influences sludge decomposition indirectly by influencing soil aeration under saturated moisture conditions. Anaerobiosis with an accompanying large decrease in the rate of sludge decomposition, an increase in odor, and reducing conditions were readily apparent in water saturated fine textured soils.

4. Soil temperature markedly affects the rate of sewage sludge decomposition in soil. A relationship between per cent sludge carbon evolved as CO_2 and temperature, expressed as Monthly Degree Days should be useful in predicting the magnitude of sludge decomposition in a given season or climatic area.

5. A comparison of population changes for fungi and bacteria in response to sludge amendments would indicate that both groups of microorganisms can participate in sludge decomposition. The dominant group under a particular set of environmental conditions will depend on the soil moisture content and a degree of soil aeration.

6. The population of total coliforms, fecal coliforms and fecal streptococci added with the sludge decreased rapidly with time. However, low numbers of total coliforms and fecal streptococci were still detected in sludge amended soils after 6 months incubation.

7. A total of 354 bacterial isolates from the Ottokee sand and Celina silt loam soils were extensively characterized using morphological and biochemical characters. The bacteria from sludge amended soils had an increased tendency to be gram negative, smaller, produce pigments, have a faster growth rate, grow at higher salt concentrations, to be more resistant to antibiotics, and to be less active in biochemical transformations.

8. Accumulation of soluble salts and soluble nitrogen in sludge amended soils are the two factors most likely to limit the rate at which sewage sludge may be added to soils.

RECOMMENDATIONS

Futher studies should be carried out on the rate of sludge decomposition with repeated small loadings, rather than with one massive loading such as was employed in this study. Rates of decomposition should also be determined for surface applied sludge versus that incorporated with the soil and filter cake sludge vs liquid sludge vs freeze dried sludge. Continued survival of low populations of indicator bacteria for periods up to 6 months in sludge amended soils suggests that further studies are needed on the survival of bacterial pathogens in soil. Even more of an unknown is the fate of enteric viruses associated with digested sewage sludge. Further studies should delineate the methodology for investigating the survival of enteric viruses in soils.

The large accumulation of soluble nitrogen in sludge amended soils points out the need for further studies on ways to accelerate losses of nitrogen during handling, storing and applying sludge to land. Such losses of nitrogen would greatly improve the suitability of sludge for disposal on land.

INTRODUCTION

Interest in land disposal of sewage sludges and primary and secondary effluents from treatment of municipal and industrial wastes is growing rapidly. The extent of this interest might be surmised by the increased generation of literature and reports on this subject (Hinesly and Sosewitz, 1969; Ewing and Dick, 1970; Hinesly et al, 1971; CRREL Special Report 171, 1972). To some, this approach to waste treatment or ultimate disposal may seem like a new concept, but in actuality the disposal of human wastes on land is as antiquated as man himself. Land disposal of municipal wastes after some degree of sanitary treatment has been employed by many European cites on a continuing basis for 50-100 years. In this country a surprising number of smaller communities have used land for disposal of sewage sludges and effluent for many years with varying degrees of success (anonomyous, 1962).

The primary reason for the attractiveness of land disposal of liquid sludge can probably be summed up in one word--economics. Recent cost evaluations have shown the cost for disposal of liquid digested sewage sludge on land within reasonable proximity to the treatment plant is usually far less than any other type of disposal method e.g. drying beds, lagoons, or incineration (Ewing and Dick, 1970; Burd, 1968). A secondary reason for increased use of land disposal for liquid sludge is that soil provides an alternate approach to the use of our already stressed air and water resources for sludge disposal.

The studies funded by this contract have considered in some detail the microbiological aspects of sewage sludge disposal in soil. The significance of studies of this type can be shown by a brief evaluation of the number of potential problems in land disposal of sewage sludge which are related to microbial activity. One problem associated with management of soils for sludge disposal is the rate at which sludge organic carbon compounds are decomposed in soil. Accumulation of organic matter in soil can have both beneficial and detrimental effects on the physical and chemical properties of soils. Information on rates of sludge degradation can therefore be useful in modifying loading rates and management to achieve the desired accumulation of organic matter. Microbial activity during sludge decomposition also modifies the mobility and solubility of inorganic compound and ions in soil by processes of mineralization, immobilization, oxidation, reduction, and chelation reactions. The impact of microbial reactions are particularly significant in modifying the rate of sewage nitrogen by the processes of ammonification, nitrification, and biological denitrification. One of the long term problems with sludge disposal on land is the accumulation of phytotoxic levels of heavy metal ions. Again, both the mobility and plant uptake of these ions are influenced greatly by microbial activity and the processes listed above. Unless treated, sewage sludge may contain relatively large numbers of pathogenic bacteria and viruses. Application of sewage sludge to agricultural lands or to lands being reclaimed must not pose a health hazard to the surrounding community. Again the normal microbial population of soil is involved in the rapid die back of these pathogens and information on these reactions is of considerable significance. Finally, microorganisms have been known to produce numerous phytotoxins, most often when supplied with high levels of organic substrate. Because the additions of sewage sludge to soil will increase the substrate level in soils, information must be obtained on this potential problem.

This contract has addressed itself in varying detail to the problems listed above. It is hoped that the information provided will increase the understanding of all the myriad microbial reactions which influence the success of a system for land disposal of sewage sludge.

MATERIALS AND METHODS

An overview of the research plan for this study is shown in Table 1. The experimental variables included three soils, Ottokee sand, Celina silt loam, and Paulding clay; two sludge amendments, 90 tons (metric)/ha (40 tons/acre) and 224 tons (metric)/ha (100 tons/acre) on a dry weight basis; two soil moisture contents, field capacity (1/3 bar moisture %) and saturation; and temperature. Temperatures were programmed within an environmental control room to provide both diurnal and seasonal temperature variation as shown in Table 2. Temperatures were maintained at the minimum and maximum temperatures for 12 hours each day for one month before changing to the next temperatures. The duration of each experiment is shown by the horizontal lines beneath the temperature data of Table 2 and corresponded to autumn-winter (Exp. I), winter-spring (Exp.II), spring-summer (Exp.III) and summer-autumn (Exp. IV) temperatures.

Detailed descriptions of the materials and methods employed will be provided in the following paragraphs.

SOILS

The three soils used in this study were chosen because they represented the extremes in soil texture i.e. sand, silt and clay. Selected physical and chemical properties are shown in Table 3. Bulk samples of each soil were obtained from the 2-15 cm depth of each soil profile, sieved through a 5 mm screeen to remove stones and plant debris, mixed and stored at 4°C until needed.

The Paulding clay and Celina silt loam soils were obtained from agricultural land while the Ottokee sand was from a coniferous-hardwood forest site.

SEWAGE SLUDGE

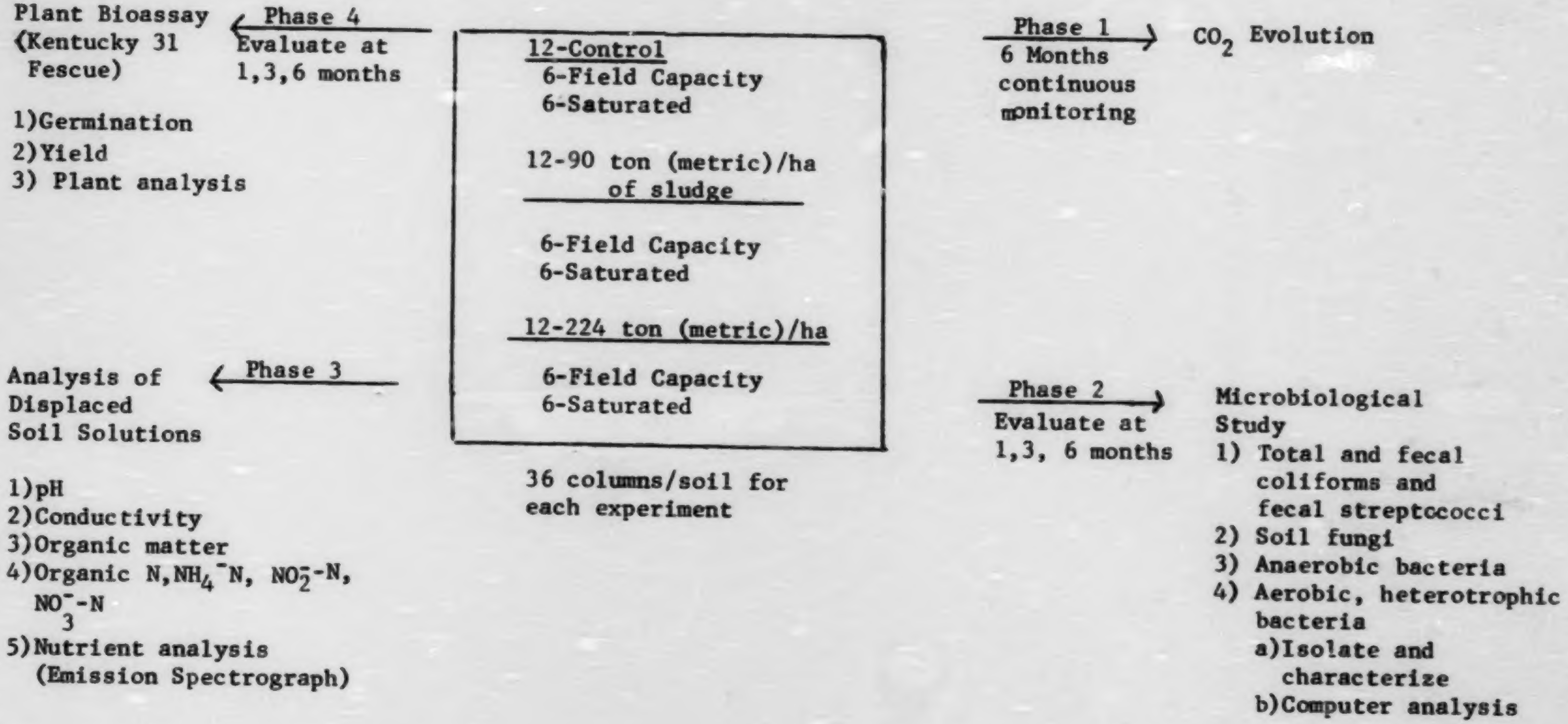
Anaerobically digested sewage sludge was obtained from the Jackson Pike Treatment Plant, Columbus, Ohio. A partial analysis of the liquid sludge at three selected time periods are shown in Table 4.

Incorporation of liquid digested sewage sludge with soil in a single application at the rates used in this study (90 and 224 ton (metric)/ha) proved a difficult task. For this reason all sludge was freeze dried prior to incorporation with the soil. The assumptions made when using this approach was that freeze drying would not chemically alter the sludge organic compounds nor influence the rate at which the microbial population would decompose them once the sludge was incorporated with the soil. Freeze drying may have altered the physical properties of the sludge organic colloids, but the changes were considered similar to those which would occur when sludge is dried in drying beds or after field spreading.

Prior to freeze drying, fresh anaerobically digested sewage sludge was concentrated to a slurry in vacuo at 40°C with a flash evaporator. The slurry was frozen immediately in 30 x 43 mm stainless steel trays. Trays containing the frozen sludge were transferred to the tray drying

Table 1. RESEARCH PLAN: MICROBIOLOGY OF A SEWAGE SLUDGE DECOMPOSITION IN SOILS.

Variables: 1) Soil properties 2) Rate of sludge amendment 3) Soil moisture content.
4) Temperature cycle



Plant Bioassay
(Kentucky 31
Fescue)

1)Germination
2)Yield
3) Plant analysis

Phase 4
Evaluate at
1,3,6 months

12-Control
6-Field Capacity
6-Saturated

12-90 ton (metric)/ha
of sludge

6-Field Capacity
6-Saturated

12-224 ton (metric)/ha

6-Field Capacity
6-Saturated

36 columns/soil for
each experiment

Phase 1
6 Months
continuous
monitoring

CO_2 Evolution

Analysis of
Displaced
Soil Solutions

1)pH
2)Conductivity
3)Organic matter
4)Organic N, NH_4^-N, NO_2^--N,
NO_3^--N
5)Nutrient analysis
(Emission Spectrograph)

Phase 3

Phase 2
Evaluate at
1,3, 6 months

Microbiological
Study
1) Total and fecal
coliforms and
fecal streptococci
2) Soil fungi
3) Anaerobic bacteria
4) Aerobic, heterotrophic
bacteria
a)Isolate and
characterize
b)Computer analysis

Table 2. TEMPERATURE PROGRAM FOR SLUDGE DISPOSAL STUDY (AVERAGE MAXIMUM AND MINIMUM TEMPERATURES (°F) ARE THOSE AT THE UNIVERSITY FARM, O.S.U., COLUMBUS, OHIO DATA 1894-1965).

Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.
Avg. Max.											
78.2	66.3	51.3	39.4	37.8	39.0	49.9	61.7	72.8	81.6	85.5	83.8
Avg. Min.											
54.6	43.2	33.6	24.8	22.2	22.7	31.2	40.5	50.3	59.3	63.0	61.3

EXP. I AUTUMN- WINTER

EXP.II WINTER-SPRING

EXP.III SPRING-SUMMER

EXP. IV AUTUMN

EXP. IV SUMMER -

Table 3. PHYSICAL AND CHEMICAL PROPERTIES OF EXPERIMENTAL SOILS

Soil	Texture %			Organic C %	Organic N %	pH	Moisture %		
	Sand	Silt	Clay				0 Bar	1/3 Bar	15 Bar
Ottokee Fine Sand	95.5	1.3	3.2	0.5	0.025	7.1	20.0	3.1	2.6
Celina Silt Loam	17.9	63.9	18.2	1.6	0.080	7.0	35.0	21.7	9.6
Paulding Clay	14.7	39.7	45.6	2.0	0.158	5.7	62.0	32.9	18.3

Table 4. ANALYSES OF DIGESTED SEWAGE SLUDGE FROM COLUMBUS, JACKSON PIKE PLANT (THREE SAMPLING TIMES) ALL VALUES ARE GIVEN AS mg/1.

	April 5, 1970	April 20, 1970	April 27, 1970
Total N	1316	1395	1234
Ammonia N	357	464	536
Total Solids	47400	49100	35500
Ash	21100	20400	17130
pH	7.3	7.0	7.1
Grease	4790	4820	---
P	1670	1460	1180
K	120	120	120
Ca	3340	2630	2240
Mg	1020	870	240
Na	280	290	150
Mn	10	11	7
Fe	52	49	67
B	6	4.8	3.5
Cu	33	34	10
Zn	84	75	58
Al	509	453	215
Si	1280	1070	2035
Ni	1.1	0.9	0.6
Cd	0.5	0.3	0.2
Pb	4.2	3.4	2.8

Table 5. ANALYSIS OF FREEZE DRIED ANAEROBICALLY DIGESTED SEWAGE SLUDGE.

Exp. #	Organic C %	Organic N %	Free NH_3 %	C:N %	Hexane Soluble %	Methanol Soluble %	Volatiles %	Ash %	H_2O %
I	25.7	3.2	0.17	7.6	8.9	4.5	52.9	47.1	4.3
II	26.5	2.9	0.21	8.5	8.3	4.7	52.9	47.1	5.5
III	26.3	2.9	0.32	8.2	8.6	4.5	51.3	48.7	4.0
IV	25.1	3.1	0.23	7.5	7.8	4.5	50.1	49.9	3.9
$\bar{X}$ =	25.9	3.0	0.23	8.0	8.4	4.5	51.8	48.2	4.4

chamber of a Virtis Freeze Mobile for freeze drying. Analyses of the freeze dried sludge as used in the four basic experiments of this study are shown in Table 5. It can be seen from these data that the carbon and nitrogen components remained remarkably constant, even though these analyses were of sewage sludge obtained periodically over a two year period.

PHASE 1: MEASUREMENT OF CO_2 EVOLUTION

Carbon dioxide evolution from sludge amended soils was measured for periods up to six months and used to determine the rate of decomposition of sludge organic matter and as an indirect measure of microbial activity.

The basic apparatus for measuring CO_2 evolution was patterned after that previously described by Stotzky (1965). Compressed air was delivered from a pressure regulator to a scrubber system consisting of concentrated sulfuric acid, 4N NaOH, and distilled water in series; passed through a glass gassing manifold with capillary tubing attached to equalized air flow and over the soil contained within plastic columns; then to a glass bead bubble tower CO_2 collectors containing 1N NaOH.

The plastic columns which contained the soil were constructed from 20° mm lengths of 8 mm (I.D.) high impact styrene pipe. The bottom of each column was sealed with a sheet of 6 mil polyvinylchloride (PVC) held in place with Scotch Brand Super strength Adhesive and a few wraps of vinyl electric tape. A No.14 rubber stopper containing glass air inlet and outlet tubes 7 mm (I.D.) and 10 mm (I.D.) glass center tube was used to close the top of each column. The center tube, used periodically to add H_2O during the incubation, was closed with a rubber stopper.

All connections of the components of the CO_2 apparatus were made with tygon tubing. Each scrubber system serviced two manifolds with 14 outlets in each. Two of the 14 outlets were used for control columns which did not contain soil. The assembled apparatus including manifold, columns and bubble towers are shown in Figure 1.

At the beginning of each experiment sufficient soil and freeze dried sludge for all replications of a given treatment were mixed within a twin shell blender for 30 minutes. Soil or soil-sludge mixtures equivalent to 600 g of oven dry soil were added to each column and packed gently by tapping. Distilled water was then added to the soil columns to bring them to the desired moisture content and the columns closed with the rubber stopper assembly described previously. All columns were then transferred to the environmental control room and connected to the CO_2 apparatus.

Tumblers of the CO_2 collectors were placed regularly as needed with fresh 1N NaOH. Since microbial activity varied considerably during the different incubation periods, the length of time between changes varied between one day and two weeks. Evolved CO_2 absorbed within the CO_2 collectors was determined by titration using a Beckman Model K Automatic Titrator after precipitation of the carbonate with Ba Cl_2.

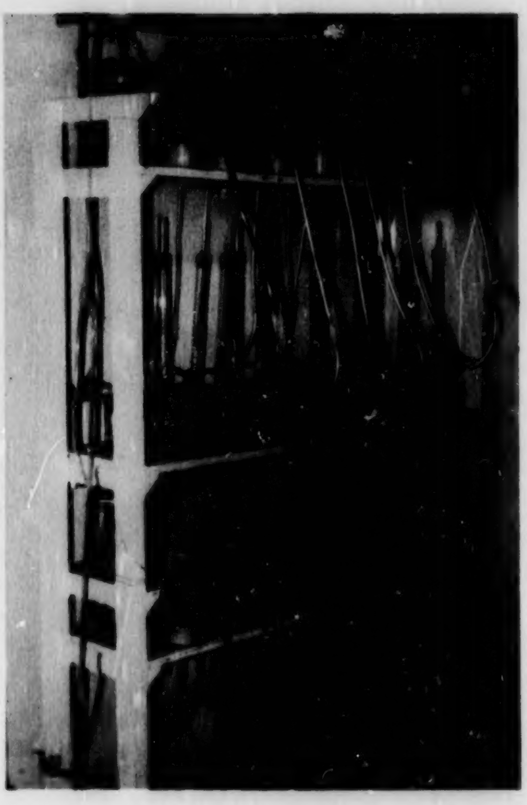


Figure 1. Assembled CO_2 train with manifold, soil columns, and bubble tower CO_2 collectors.

After 1, 3 and 6 months incubation duplicate columns of each soil were removed from the CO_2 apparatus and used for the analyses listed under Phases 2, 3 and 4 (Table 1). The soil samples removed from the columns were thoroughly mixed, transferred to plastic lined cardboard cartons (86 x 165 mm), and stored at 4° C until analyzed. In most cases the platings for the microbiological study, the displacement of the soil solution, and platings for the plant bioassay were all completed within one week. Storage at 4°C was utilized to minimize microbial changes before analysis.

PHASE 2: MICROBIOLOGICAL STUDY

Population estimates for a number of significant groups of microorganisms in the sludge amended soils were made routinely after 1, 3 and 6 months incubation. An attempt was made to perform all determinations within a few days after the soil was removed from the columns to minimize changes in the microbial population because of storage.

Population estimates of coliforms and fecal streptococci in sludge amended soils were made from appropriate soil dilutions using membrane filter techniques as described in Standard Methods (American Public Health Association, 1965). Fecal coliforms were estimated by the high temperature membrane filter technique developed by Geldreich et al (1965). Fungi were estimated by dilution plating on rose bengal-streptomycin agar (RBS) (Martin, 1950). All plates were incubated for 7 days at 26°C before counting.

Aerobic heterotrophic bacteria including actinomycetes were determined by spreading 0.1 ml of the desired dilutions on pre-poured plates of sludge-soil extract agar (SS)(See Appendix A.). All plates were incubated for 7 days at 26° C before counting. Anaerobic bacteria were also enumerated on sludge-soil extract agar plates incubated in Gas-Pak Anaerobic jars for two weeks at 26°C. Individual colonies of aerobic heterotrophic bacteria for subsequent detailed characterization were isolated from sludge-soil extract agar plates prepared from soils after 1 months incubation. The isolated colonies were transferred into screw capped nutrient agar slants and stored at 4°C. When possible the individual colonies selected were taken from dilution plates containing 10 colonies or less. Where this approach was not applicable, 10 colonies were selected at random from the highest dilution series employed. All cultures were transferred bi-monthly during the period of characterization.

Each of the cultures was examined for the morphological, colonial, physiological and biochemical characters listed in Table 6. In preparation for the various tests, stock cultures were transferrred to flasks of nutrient broth. The inoculated flasks were shaken gently on a gyrotory shaker until visible turbidity developed. These actively growing cultures were then used for microscopic examination as well as for the source of inoculum for the various test media. Standard techniques and media were employed for the various biochemical tests (Collins & Lyne, 1970). Physiological evolutions such as growth rate, temperature range, and growth in NaCl were made on nutrient broth or nutrient agar. Acid production from various carbohydrates was determined using the miniaturized Microtiter technique of Fung and Miller (1970). Acid production under

Table 6. CHARACTERS FOR WHICH ALL BACTERIA WERE EXAMINED

Character	No. of States
Cell shape	5
Cell length	3
Cell width	3
Presence of:	
-spores	2
-plemorphism	2
-filaments	2
Cell arrangement	4
Gram Stain	3
Colony-size	3
" -shape	8
" -pigmentation	9
Type of growth in broth	4
Relative growth	3
Mobility	2
Thermotolerance	3
Salt tolerance	3
Resistance to:	
Viomycin	2
Streptomycin	2
Chloromycetin	2
Novobiocin	2
Bacitracin	2
Tetrocycline	2
Penicillin	2
Hydrolysis of:	
-cellulose	2
-starch	2
-pectin	2
-gelatin	2
-tributyrin	2
-triolein	2
Production of:	
-catalase	2
-cytochrome oxidase	2
-urease	2
-indole	2
-acetylmethylcarbinol	2
-acid (Methyl-red)	2
-hydrogen sulfide	2
Utilization of citrate	2
Litmus milk	7
Reduction of nitrate	4
Production of acid from:	
-glucose *	4
-fructose*	4
-mannose *	4
-galactose *	4
-arabinose *	4
-ribose	2
-lactose *	4
-sucrose *	4
-maltose *	4
-cellobiose *	4
-raffinose	2
-dextrin	2
-inulin *	4
-mannitol	2
-sorbitol*	4
-dulcitol *	4

* Evaluated both aerobically and anaerobically.

anaerobic conditions also utilized the microtiter plates maintained in Gas-Pak jars for a two week period. Incubation temperatures were 26° C except for the temperature studies and gelatin hydrolysis. Time of incubation was variable for the various tests and took into account the differing growth rates of the isolates.

Upon completion of the analyses, data were recorded on IBM punched cards as a 1 for the presence of a character or a positive test, or as a 0 for the absence of a character or a negative test. Inapplicable characters or tests not done were scored as a 3. All evaluations for each character were expressed on a percentage basis by the use of a computer program.

PHASE 3: ANALYSIS OF DISPLACED SOIL SOLUTIONS

Soil solutions were displaced from the sludge amended and control soils after 1, 3 and 6 months incubation. Solutions were obtained from the Ottokee sand and Celina silt loam soils by a liquid displacement method using 50% glycerol similar to that used by Larson and Widdowson (1968). Moist soil, equivalent to 325 g of oven-dry soil, was mixed with 100 g of acid washed quartz sand, and added stepwise with tamping to glass columns. The columns were constructed of 100 x 2.5 cm lengths of pyrex tubing closed at one end with a rubber stopper and 6 cm length of 7 mm (I.D.) glass tubing. Glass wool was placed in the bottom of each column to retain soil particles. Initial studies indicated that approximately 50% of the soil water could be collected without contamination with glycerol. The time necessary to displace the desired quantity of soil solution varied from about 1 hour in the Ottokee sand to as long as 12 hours with some samples of the Celina silt loam soil. Positive pressure was applied in some instances to increase the flow rate through the column. The volume of all solutions was measured and the solutions transferred to polyethylene bottles for storage at -20° C. All solutions were thawed rapidly in hot water before analysis. The liquid displacement method was not effective in obtaining soil solutions from Paulding clay because of very slow flow rates through the column. Soil solutions from this soil were obtained by use of a pressure membrane at 15 atms. pressure (Reitemeir and Richards, 1944). The pH of the soil solutions was measured with a glass electrode using a Corning Model 10 pH Meter. Specific conductance (mmhos/cm) was measured using an Industrial instruments, Model RC 16B2 Conductivity Bridge. The cell constant for the conductance cell was 1.03.

Organic matter was determined colorimetrically after oxidation of the organic matter with a 0.15 N potassium dichromate-sulfuric acid solution. The resultant green color was read in a Coleman Universal Spectrophotometer at 645 nm (Carolan 1948).

Organic nitrogen was measured by a micro technique described by Umbreit et al (1964). The digestion was carried out in 18 x 15 mm test tubes placed within a heating block using a digestion mixture of 1 ml of 2N sulfuric acid containing copper sulfate and sodium selenite. Ammonium nitrogen in the digests was determined by Nesslerization.

Nitrate, nitrite and ammonium nitrogen were determined by the steam distillation methods of Bremmer (1965).

Direct analysis of P, Ca, K, Mg, Na, Mn, Fe, Al, Zn, Cu, Mo, and B were carried out on 5 ml aliquots of the soil solutions by use of a Jarrell-Ash Model 66-000 direct reading **Emission Spectrograph.**[1]

PHASE 4: PLANT BIOASSAY (KENTUCKY 31 FESCUE)

Control and sludge amended soils were removed from the soil columns after 1, 3 and 6 months and sub-samples used to evaluate the effect of sewage sludge on the germination, and dry matter yield of Kentucky 31 Fescue (*Festuca arundinacea*) Fescue was chosen as the bioassay plant because it is hardy and will grow reasonably well under saturated moisture conditions.

Soil from each column equivalent to 225 g of oven dry soil was weighed into 12 oz. waxed cardboard containers and compacted gently. Seventy five seeds of Kentucky 31 Fescue were spread uniformly over the surface of the soil in each container, and covered with 10 g of exfoliated vermiculite. Immediately after planting all of the containers were transferred to a growth chamber programmed for a 16 hour light period, a day temperature of 30°C and a night temperature of 23° C. The plants were watered daily with distilled H_2O to maintain them at field capacity or soil saturation.

Germination was evaluated 3 weeks after planting by counting the number of developing seedlings. Dry matter yield of the top growth was determined 6 weeks after planting on plant material dried at 70° C for 24 hours.

Plant samples were ground in a stainless steel Wiley Mill and subsamples used for plant analysis. Potassium, phosphorus, calcium, magnesium, iron, sodium, silicon, manganese, strontium, barium, boron, copper, zinc, molybdenum and aluminum were determined by a direct reading Jarrell-Ash Model 66-000 Emission Spectrograph using the standard using the standard techniques employed by the Ohio Plant Analysis Laboratory. Total nitrogen was determined using the Technicon Kjeldahl Nitrogen Apparatus and modification of the procedure of Ferrari. Sulfur was determined on perchloric-nitric acid digests using a $Ba\ SO_4$ turbidimetric technique.

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[1] Ohio Plant Analysis Laboratory, Wooster, Ohio

RESULTS AND DISCUSSION

PHASE 1: MEASUREMENT OF CO_2 EVOLUTION

Anaerobically digested sewage sludges contain about 25% organic carbon on a dry weight basis (Burd, 1968), See also Table 5. During the process of anaerobic digestion the waste organic solids are stabilized by the almost complete microbial fermentation of carbohydrates (the exception is cellulose) resulting in a 60-75% reduction in volatile solids. Although data on the organic analysis of anaerobically digested sludge is difficult to obtain the residual organic material consists of a mixture of microbial tissue, lignin, cellulose, lipids, organic nitrogen compounds, and humic acid-like materials (McCoy, 1971).

At the present time there is a limited amount of data on the decomposition of anaerobically digested sludge organics in soil. Thomas and Bendixen (1969) studied the rate of decomposition of organic materials of septic tank effluent and secondary effluent in sand and soil lysimeters. Data from these studies indicated that these waste organics are readily biodegradable (67-89%) with little effect of temperature, loading rate, or duration on the rate of degradation. The authors used their data to conclude that sewage sludges might be expected to degrade in a similar manner and thus no detrimental accumulation of organic residues would occur. It is doubtful, however, if this conclusion will be valid for anaerobically digested sludges which have a much lower percent volatile solids than the waste materials used by Thomas and Bendixen.

A recent report by ARS personnel, Beltsville, Maryland (1972) includes studies on the biodegradation of a number of sludges including a digested sludge. Less than 10 percent of the carbon from digested sludge added to soil was evolved as CO_2 when incubated at 26° for 54 days. At the same time "raw" sludges showed an average loss of 27 percent carbon. These studies would seem to provide data more in line with the expected biological stability of anaerobically digested sludges.

The studies in this section of the report were designed to provide additional information on the rate of biodegradation of digested sludge and to evaluate what influence soil properties, loading rate, soil moisture content, and temperature would have on this microbial activity. In the case of the temperature variable an attempt was made to duplicate natural conditions by providing for both a diurnal as well as seasonal temperature changes during the course of the study. Some recent reports have pointed out that constant temperature studies of microbial activity in soils are not useful in extrapolating laboratory data to actual field conditions.

One of the more significant results from this study was the observation that the rate at which the organic carbon of digested sewage sludge was evolved as CO_2 by microbial activity was largely independent of soil properties. The obvious exception is soil moisture as influenced by soil texture or soil structural features which will continue to have a marked influence on water availability or soil aeration. These considerations will be discussed in subsequent paragraphs. The data in Figures 2, 3, 4, and 5 are cumulative plots of CO_2 evolution from all three

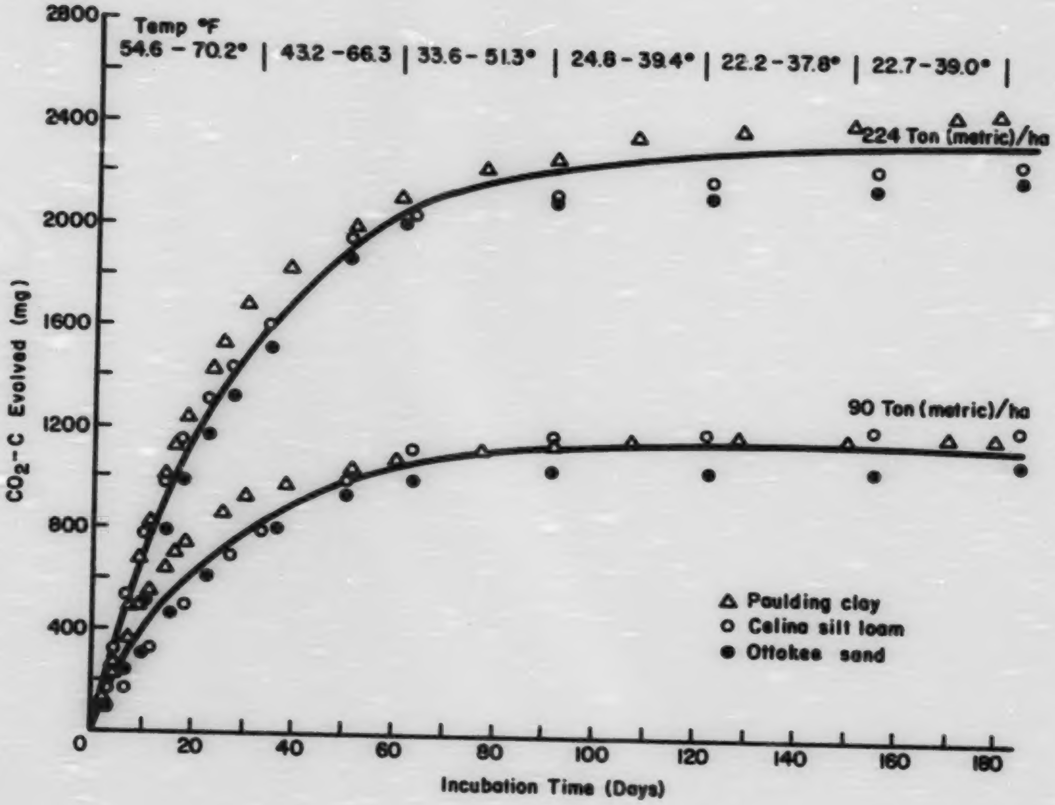

Figure 2. Cumulative plot of CO_2-C evolution from sludge amended soils during temperatures equivalent to autumn-winter (Columbus, Ohio).

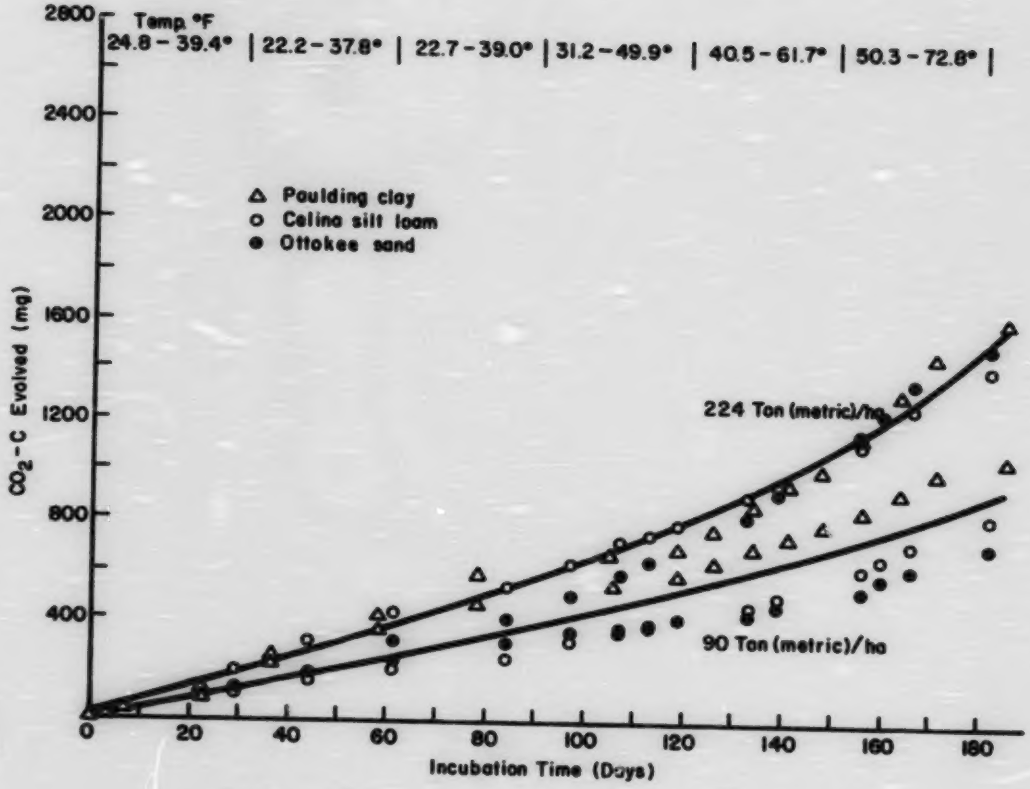

Figure 3. Cumulative plot of CO_2-C evolution from sludge amended soils during temperatures equivalent to winter-spring (Columbus, Ohio).

sludge amended soils over 6 month periods equivalent to autumn-winter, winter-spring, spring-summer, and summer-autumn temperatures in Columbus, Ohio. These data show that except for some unexplainable deviations by the sludge amended Paulding clay (Figures 4 and 5) the correspondence between soils with respect to CO_2 evolved was excellent. In practical terms this means that at the rather high sludge loading rates employed in this study (90 and 224 metric tons/ha) the initial soil properties which would influence microbial activity are effectively masked, and the sludge soil system is actually behaving as a sludge system. The significance of this observation is that if soil moisture is neither restrictive or excessive, anaerobically digested sewage added to low fertility or marginal soils for renovative purposes could be expected to decompose at the same rate as when applied to more fertile agricultural soils.

The above data on the independence of sludge decomposition to soil properties made it feasible to attempt to plot sludge decomposition as a function of total heat imput to the soil-sludge system. The degree day concept (see equation 1 below) was chosen as a useful indicator of heat imput.

$$1)\ \text{Monthly Degree Days} = \sum_{i=i}^{N} \left(\bar{\bar{X}}_{MT} + \bar{\bar{X}}_{mt}/2 \right) \times 30$$

where $\bar{\bar{X}}_{MT}$ = mean daily maximum temperature during a month (^{o}F)

$\bar{\bar{X}}_{mt}$ = mean daily minimum temperature during a month (^{o}F)

N = number of months

The relationship between degree days and % sludge carbon evolved as CO_2 for the 90 and 224 metric ton amendments of sewage sludge is shown in Figures 6 and 7. These data show quite clearly that a highly significant correlation exists between CO_2 evolved and degree days after both one and three month incubation periods. Correlation coefficients for data obtained after 6 months incubation are not as high but still show a statistically significant relationship (0.01 probability) between temperature and CO_2 evolution. Data of this type should make it possible to predict the amount of decomposition of sewage sludge carbon per unit time in different climatic regions using existing temperature data.

These studies also provide further evidence that anaerobically digested sewage sludge is rather resistant to further biological decomposition when added to soil. A maximum of 20 percent of the added carbon was evolved as CO_2 from the 90 metric ton amendment (Figure 6) while slightly less, 18 percent, was evolved from the larger, 224 metric ton amendment (Figure 7). The decreasing slope of the lines with time in Figures 6 and 7 also shows quite clearly that most of the sludge decomposition occurs during the first months incubation. The obvious conclusion that can be drawn from these data is that the addition of anaerobically digested sewage sludge to soil will result in an increase in the level of soil organic matter.

The previous discussion implied that the decomposition of anaerobically digested sewage sludge was largely independent of differences in soil

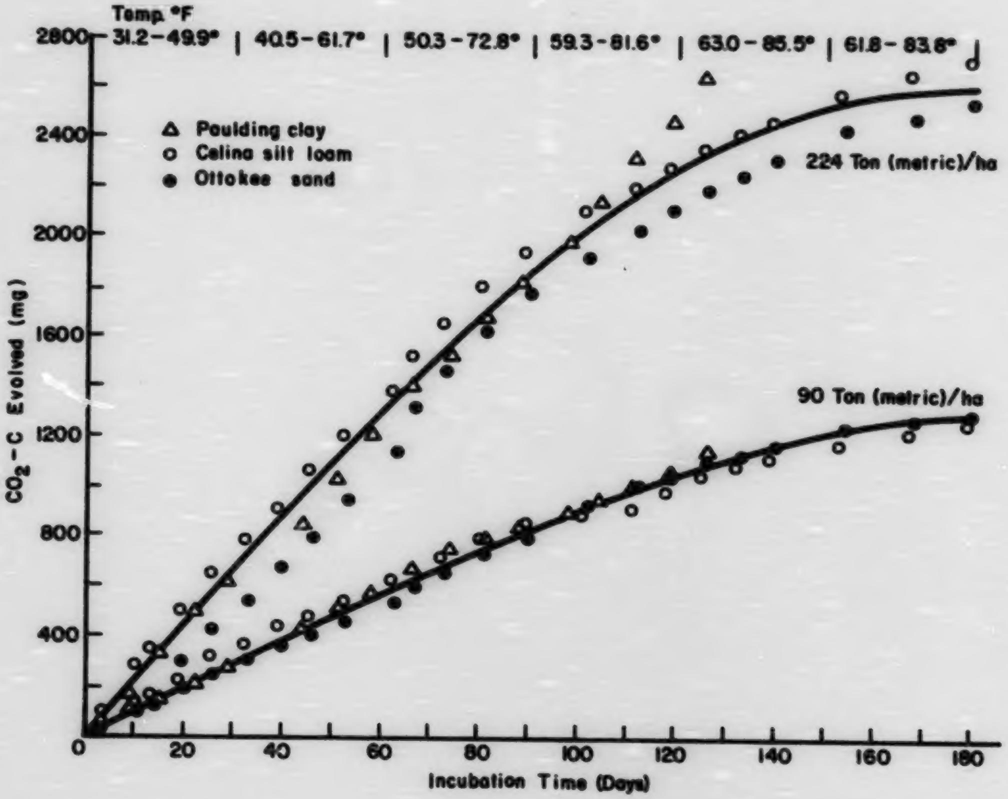

Figure 4. Cumulative plot of CO_2-C evolution from sludge amended soils during temperatures equivalent to spring-summer (Columbus, Ohio).

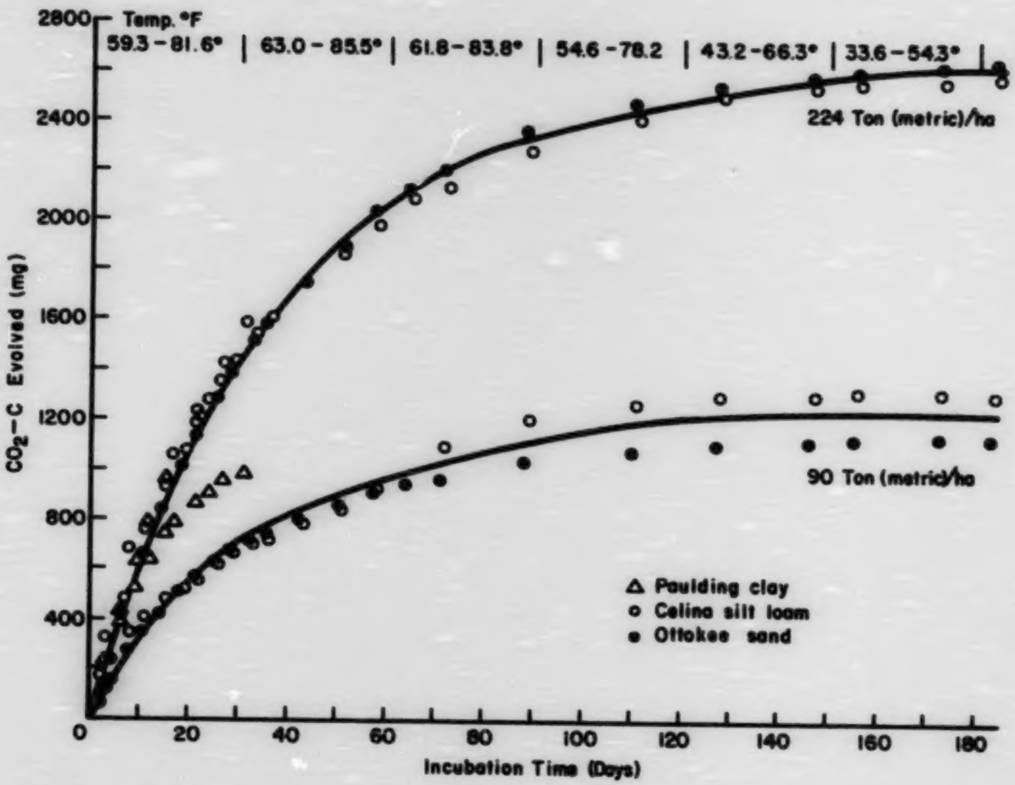

Figure 5. Cumulative plot of CO_2-C evolution from sludge amended soils during temperatures equivalent to summer-autumn (Columbus, Ohio).

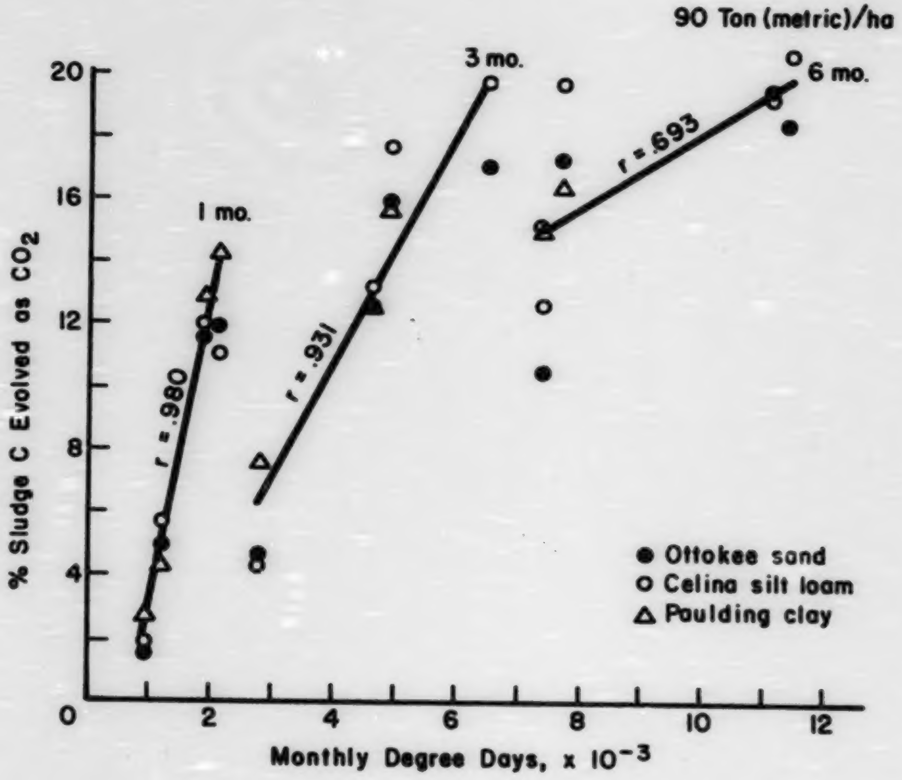

Figure 6. Relationship between % sludge carbon evolved as CO_2 and degree days, (F°), (90 metric ton/ha amendment).

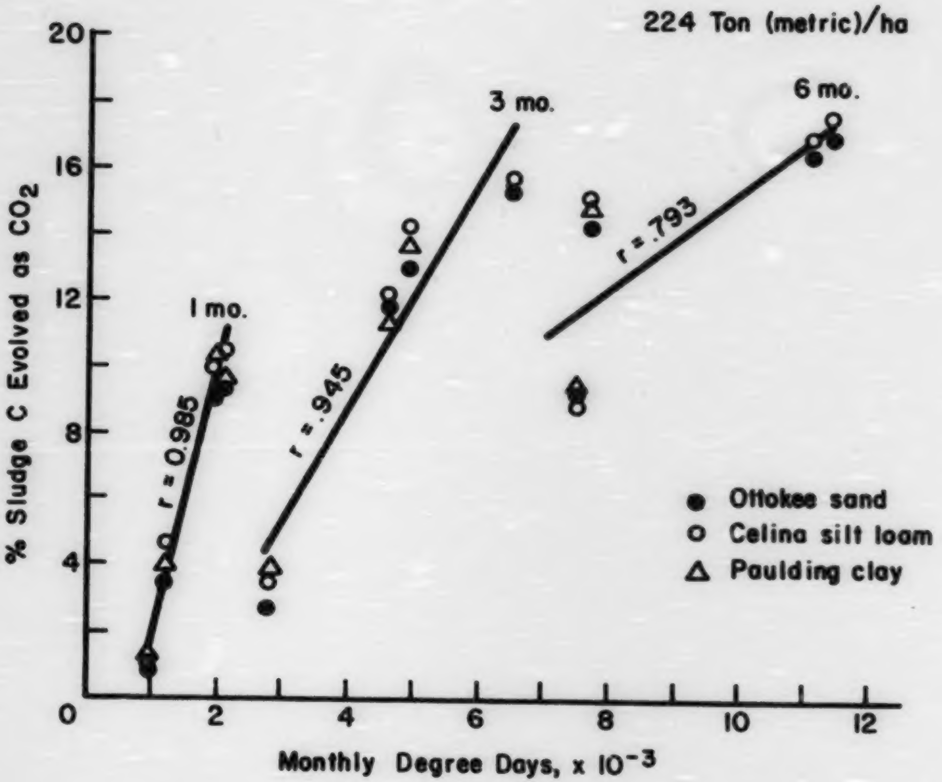

Figure 7. Relationship between % sludge carbon evolved as CO_2 and degree days, (F^o), (224 metric ton/ha amendment).

properties. One exception to this conclusion is the influence of soil texture on the degree of microbial activity under 1/3 bar and saturated moisture conditions. The influence of soil moisture on CO_2 evolution from sewage sludge amendments in the three soils for Experiment I is shown in Figures 8, 9, 10. Note that the maximum microbial activity in the Ottokee sand occurs under saturated moisture conditions at both the 90 and 224 metric ton loading rates (Figure 8). In the Celina silt loam there is no difference in CO_2 evolution from soils at 1/3 bar and saturated moisture conditions at the 224 metric ton additons, but a marked reduction occurs under saturated conditions at the 90 metric ton rate (Figure 9). With the Paulding clay saturated soil moisture conditons severly restrict microbial activity at both loading rates (Figure 10).

The data provided above suggest that in the Ottokee sand sufficient O_2 can diffuse into the soil columns at saturated moisture conditions to maintain an active microbial population. The reduction of microbial activity with the moisture content at 1/3 bar pressure is probably associated with the high soluble salt content associated with the sludge amendments which have increased the osmotic potential of the soil water to a point where it was detrimental to microbial activity (See Phase 3, Table 20). In the Paulding clay the effect of soil saturation in reducing the diffusion of O_2 into the soil columns resulting in anaerobic conditions and reduced microbial activity is apparent. The same explanation is probably valid for the Celina silt loam soil at the 90 metric ton sludge amendment. It is more difficult to explain the high microbial activity in the Celina soil under saturated conditions at the high 224 metric ton loading rate. Presumably the high osmotic potential associated with soluble salts as well as possible effects associated with the increase in organic matter have altered the activity of the water and allowed for adequate aeration. In general, the effects of soil moisture shown in Figures 8, 9, and 10 for Experiment I were consistent in all other experiments. There were slight deviations, however, the most pronounced being a more rapid decomposition of sewage sludge carbon in the Paulding soil under saturated conditions when summer temperatures were maintained. Tabular data on percent sludge carbon evolved as CO_2 for all loading rates, soils, moisture treatments and seasonal temperatures are shown in Tables 7, 8 and 9.

These data point out the obvious importance of proper management of soils to which liquid sewage sludge is applied. Saturation of fine textured soils would certainly produce a slower rate of sludge decomposition and associated problems of anaerobiosis and odor. Course textured sandy soils would be less affected by saturated moisture conditions but might suffer from periodic water deficits. Other problems associated with these soils which will be discussed later e.g. downward movement of nitrate and other soluble salts may limit their applicability as disposal sites.

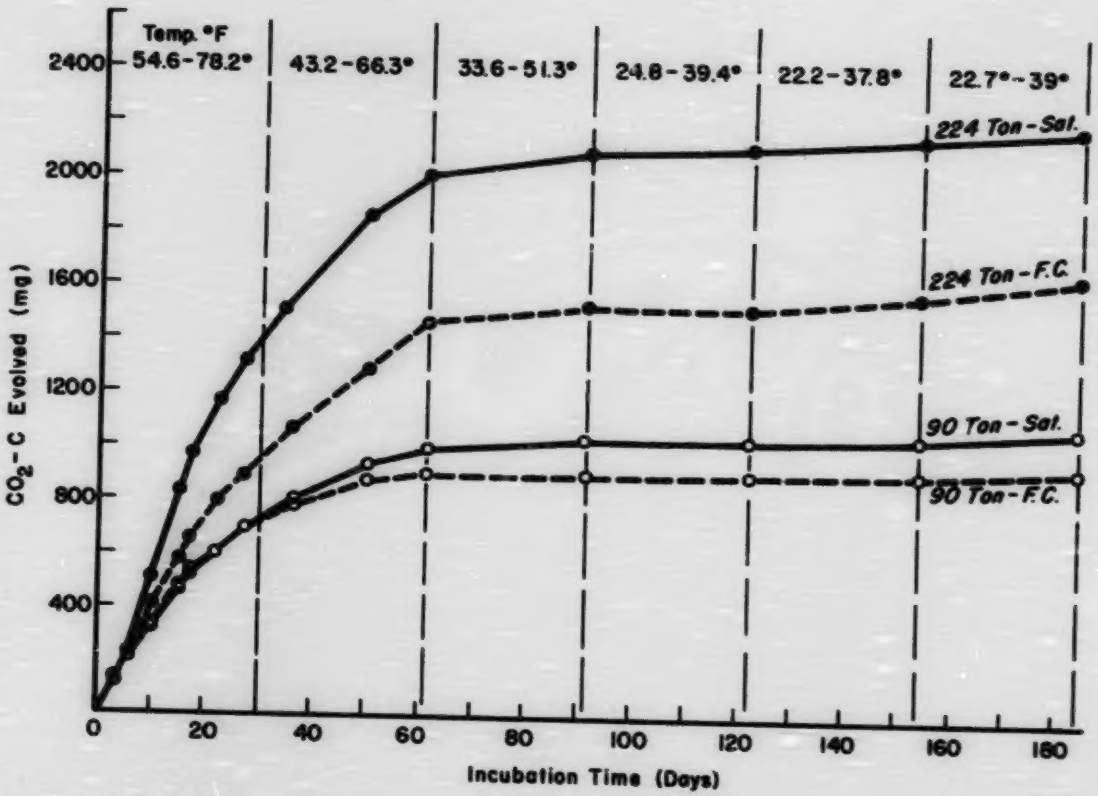

Figure 8. Cumulative plot of CO_2-C evolution from sludge amended Ottokee sand (autumn-winter temperatures, Columbus, Ohio).
Sat. = Soil saturated F.C. = Field capacity ie. moisture retained in soil under 1/3 bar pressure.

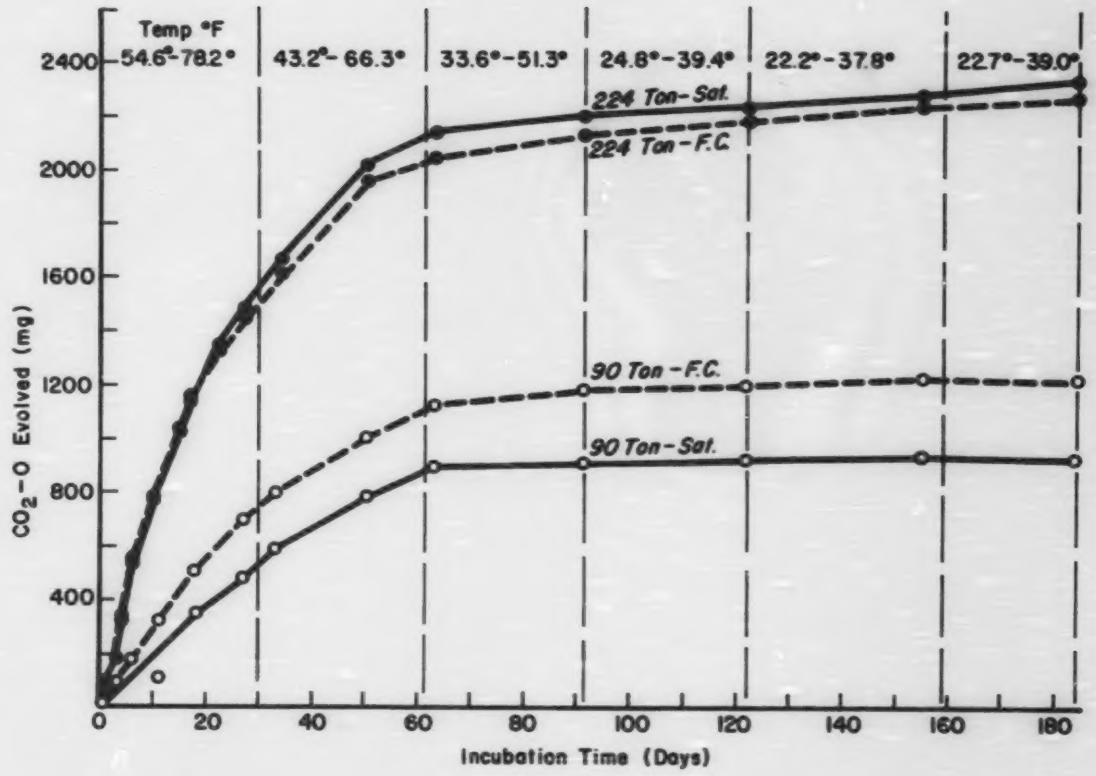

Figure 9. Cumulative plot of CO_2-C evolution from sludge amended Celina silt loam (autumn-winter temperatures, Columbus, Ohio).
Sat. = Soil saturated F.C. = Field capacity ie. moisture retained in soil under 1/3 bar pressure.

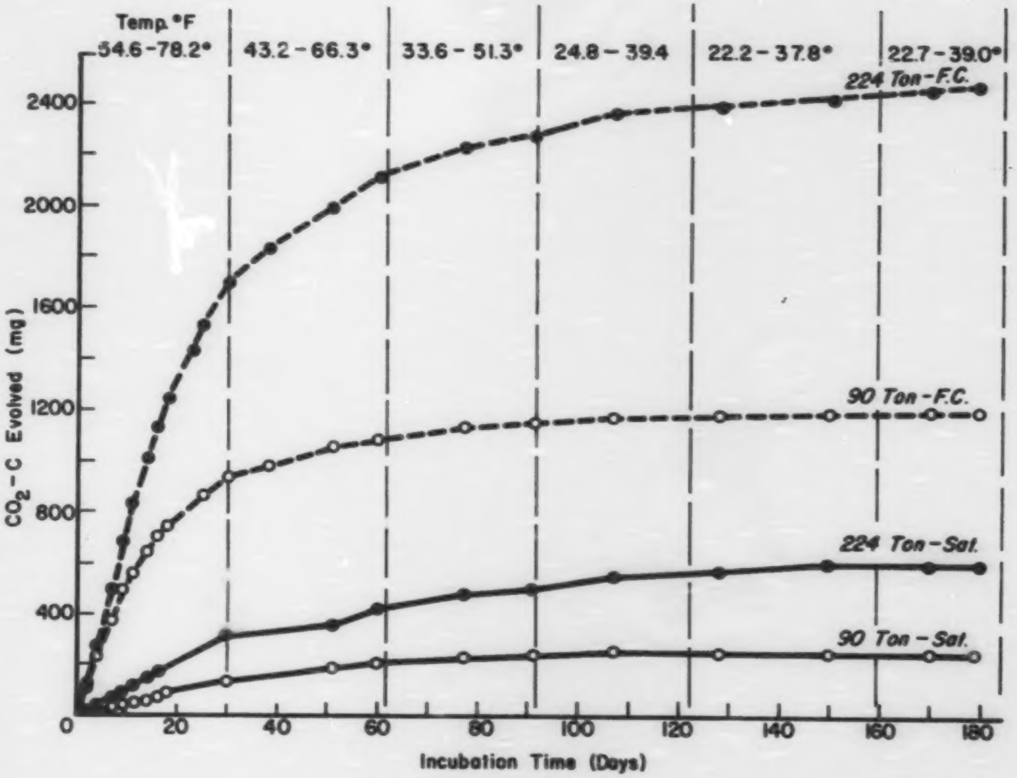

Figure 10. Cumulative plot of CO_2-C evolution from sludge amended Paulding clay (autumn-winter temperatures, Columbus, Ohio).
Sat. = Soil saturated F.C. = Field capacity ie. moisture retained in soil under 1/3 bar pressure.

Table 7. SLUDGE DECOMPOSITION IN OTTOKEE SAND AS AFFECTED BY SEASONAL TEMPERATURES

Temp. Equiv.	Treatment[a]	% of Added Carbon Evolved as CO_2 at.		
		1 Mo.	3 Mo.	6 Mo.
Autumn	90 Ton-FC	11.6	16.0	17.4
Winter	90 Ton-Sat.	11.6	14.4	14.7
Exp. I	224 Ton-FC	6.2	9.3	10.7
	224 Ton-Sat.	9.1 (9.6)[b]	13.0 (13.1)	14.3 (14.3)
Winter	90 Ton-FC	0.9	2.1	6.7
Spring	90 Ton-Sat.	1.5	4.7	10.6
Exp.II	224 Ton-FC	0.5	1.9	4.5
	224 Ton-Sat.	0.8 (0.9)	2.7 (2.9)	9.3 (7.8)
Spring	90 Ton-FC.	4.4	12.5	19.5
Summer	90 Ton-Sat.	5.0	12.6	17.7
Exp.III	224 Ton-FC	3.1	11.1	15.7
	224 Ton-Sat.	3.5 (4.0)	11.9 (12.0)	16.5 (17.4)
Summer	90 Ton-FC	11.9	17.1	18.5
Autumn	90 Ton-Sat.	11.0	16.1	17.3
Exp.IV	224 Ton-FC	8.1	15.0	17.0
	224 Ton-Sat.	9.4 (10.1)	15.4 (15.9)	17.0 (17.5)

[a] Sludge amendment in metric ton/ha. FC= field capacity Sat. = saturated moisture conditions.

[b] Numbers in ( ) represent Mean % carbon evolved for each month season combination.

Table 8. SLUDGE DECOMPOSITION IN CELINA SILT LOAM AS AFFECTED BY SEASONAL TEMPERATURES

Temp. Equiv.	Treatment[a]	% of Added Carbon Evolved as CO_2 at.		
		1 Mo.	3 Mo.	6 Mo.
Autumn	90 Ton-FC	12.0	17.7	19.7
Winter	90 Ton-Sat.	8.7	14.6	15.0
Exp. I	224 Ton-FC	9.8	13.8	14.7
	224 Ton-Sat.	10.1 (10.1)[b]	14.3 (15.1)	15.1 (16.1)
Winter	90 Ton-FC	1.8	4.4	12.7
Spring	90 Ton-Sat.	0.1	1.0	2.7
Exp.II	224 Ton-FC	1.2	3.6	8.8
	224 Ton-Sat.	1.1 (1.1)	3.2 (3.1)	8.2 (8.1)
Spring	90 Ton-FC	5.7	13.3	19.4
Summer	90 Ton-Sat.	2.8	9.8	16.7
Exp.III	224 Ton-FC	4.7	12.2	17.0
	224 Ton-Sat.	4.6 (4.5)	11.7 (11.8)	15.8 (17.2)
Summer	90 Ton-FC.	11.1	19.7	20.7
Autumn	90 Ton-Sat.	10.2	17.0	21.6
Exp.IV	224 Ton-FC.	10.5	15.4	16.7
	224 Ton-Sat.	9.7 (10.4)	15.7 (17.0)	17.7 (19.4)

[a] Sludge amendment in metric ton/ha. FC= field capacity
Sat. = saturated moisture conditions

[b] Numbers in ( ) represent Mean % carbon evolved for each month season combination.

Table 9. SLUDGE DECOMPOSITION IN PAULDING CLAY AS AFFECTED BY SEASONAL TEMPERATURES

Temp. Equiv.	Treatment[a]	% of Added Carbon Evolved as CO_2 at. 1 Mo.	3 Mo.	6 Mo.
Autumn	90 Ton-FC	12.9	15.7	16.5
Winter	90 Ton-Sat.	1.1	2.0	2.0
Exp.I	224 Ton-FC	10.2	13.7	14.9
	224 Ton-Sat.	1.8 (6.5)[b]	2.5 (8.5)	3.0 (9.1)
Winter	90 Ton-FC	2.7	7.7	15.1
Spring	90 Ton-Sat.	0.3	0.7	5.3
Exp. I	224 Ton-FC	1.2	3.9	9.4
	224 Ton-Sat.	0.3 (1.1)	0.8 (3.3)	2.9 (8.1)
Spring	90 Ton-FC	4.4	12.6	-
Summer	90 Ton-Sat.	0.1	2.1	-
Exp.III	224 Ton-FC	4.0	11.4	-
	224 Ton-Sat.	0.4 (2.2)	6.3 (8.1)	-
Summer	90 Ton-FC	14.3	-	-
Autumn	90 Ton-Sat.	3.0	-	-
Exp.IV	224 Ton-FC	9.6	-	-
	224 Ton-Sat.	8.6 (8.9)	-	-

[a] Sludge amendment in metric ton/ha. FC= field capacity
Sat= saturated moisture conditions

[b] Numbers in ( ) represent Mean % carbon evolved for each month season combination.

PHASE 2: MICROBIOLOGICAL STUDY

One of the objectives of this study was an evaluation of the influence on anaerobically digested sewage sludge on microbial activity and microbial population in soil. It was hoped that detailed studies of the bacterial population would provide information to aid in solving problems in public health, sewage sludge degradation, and the overall management of soils amended with sewage sludge. Evidence for the occurrence of specific physiological and morphological groups of microorganisms should provide a reliable indicator of the success of the disposal process and to forecast potential problems so that corrective measures can be applied. The following paragraphs will provide data which will show how well these objectives were met.

An overall evaluation of the activity of the heterotrophic microbial population was obtained from the measurement of evolved CO_2-C from sludge amended soils. Since the data and conclusions from this portion of the study are given in Phase 1 of this report, no further discussion will be provided in this section.

Numbers of aerobic heterotrophic bacteria and actinomycetes were estimated by dilution plate counts on sludge-soil extract agar. No attempt was made to separate and estimate the population of actinomycetes. Although one must be aware of the obvious weakness of the plate count technique in estimating the total population of bacteria in soil (Jensen, 1968), this technique can provide useful relative information not attainable by any other method.

Data on the numbers of bacteria and actinomycetes for all sludge loading rates, soil moisture levels, and seasonal temperatures are shown for the Ottokee sand (Figure 11), Celina silt loam (Figure 12), and Paulding clay (Figure 13). A number of general conclusions can be drawn from these data:

1) Numbers of bacteria and actinomycetes are generally directly related to the rate of sludge addition.

2) Maximum numbers of bacteria and actinomycetes were usually found after one month's incubation and the numbers decreased after 3 and 6 month's incubation. The exceptions occurred in Experiment II, where the incubation temperature during the first three months was equivalent to winter temperatures in Columbus, Ohio. This trend in population of bacteria and actinomycetes corresponds closely with the CO_2 evolution data (Figures 2-5).

3) The largest numbers of bacteria and actinomycetes for soils incubated at field capacity followed the order, Paulding clay $\gg$ Celina silt loam $>$ Ottokee sand. In saturated soils the order was reversed so that numbers in the Ottokee sand $>$ Celina silt loam $>$ Paulding clay. The latter trend was not consistent for the Paulding clay at the high sludge loading rate. The high numbers of bacteria in the Paulding clay at the 224 metric tons loading rate after three month's incubation under saturated conditions in Experiments II and III cannot be explained at this time.

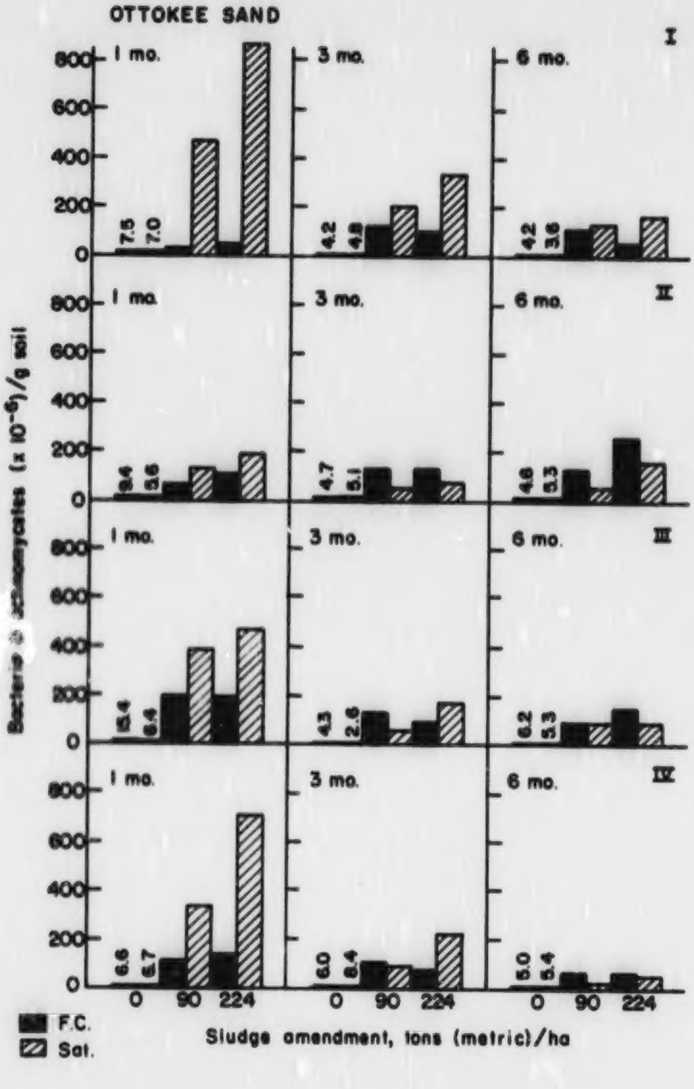

Figure 11. Dilution plate counts of bacteria and actinomycetes in sludge amended Ottokee sand as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV).

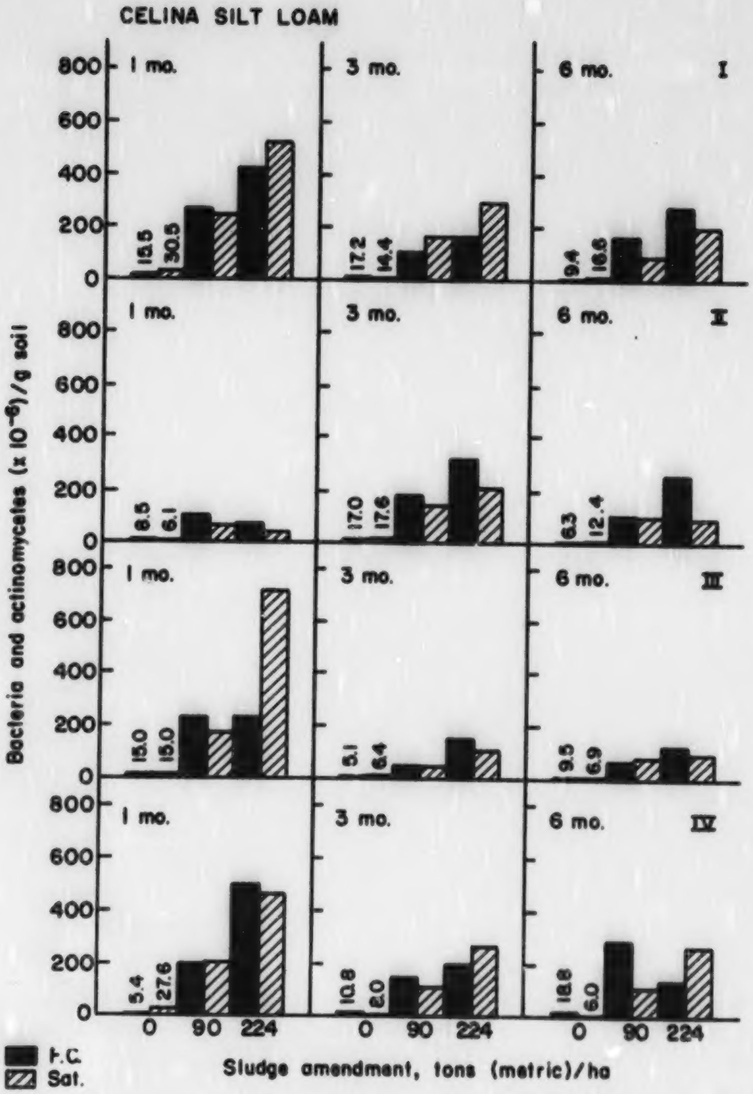

Figure 12. Dilution plate counts of bacteria and actinomycetes in sludge amended Celina silt loam as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV).

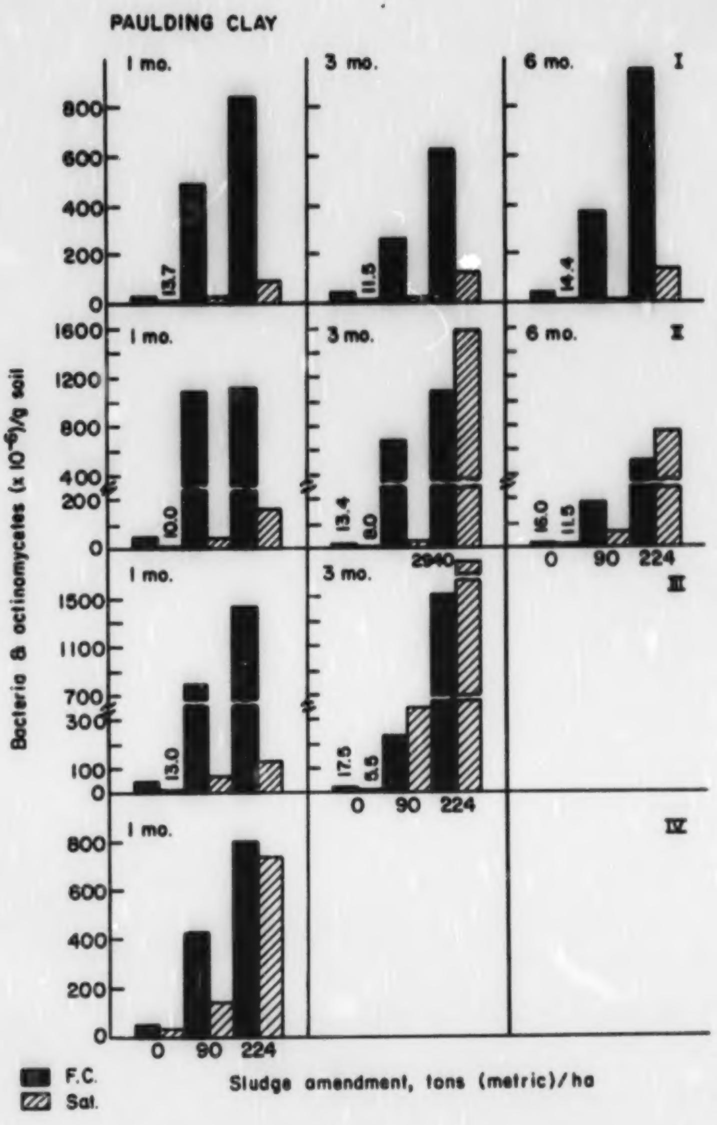

Figure 13. Dilution plate counts of bacteria and actinomycetes in sludge amended Paulding clay as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV).

4) The effect of higher temperatures in increasing microbial activity and numbers can be shown by the correspondence between numbers of bacteria and actinomycetes and incubation temperature during the first months incubation for each experiment. Average incubation temperatures followed the order Experiment $I \simeq IV > III > II$. For the actual incubation temperatures see Table 2, page 7 .

Numbers of anaerobic or facultative anaerobic bacteria in the sludge amended soils as determined by dilution plating are given in Figures 14, 15, 16. The following general conclusions can be drawn from these data:

1) The largest population of anaerobic or faculatively anaerobic bacteria was found in the Paulding clay and Celina silt loam soils with a rather small number of anaerobic bacteria found in the Ottokee sand. These results are expected, since the fine textured soils would provide more opportunity for anaerobic microsites than would a coarse textured soil such as the Ottokee.

2) The numbers of anaerobic or facultatively anaerobic bacteria increased with increasing quantities of sewage sludge. These data are as expected since large quantities of decomposable substrate should reduce the oxygen tension in microsites so that anaerobic bacteria could proliferate.

3) Also as expected, the numbers of anaerobic or facultatively anaerobic bacteria generally increased in saturated soils where oxygen diffusion would be restricted.

It is difficult to propose any significant role for the anaerobic bacteria in the decomposition of sewage sludge in soil. Generally, the population of anaerobic bacteria were less than 10 per cent of the total population of aerobic heterotrophic bacteria. This was not true, however, in the Celina silt loam soil amended with 90 metric tons of sludge in Experiment IV (temperatures equivalent to summer-autumn). In this case the anaerobic population approach 75-90 per cent of that determined under aerobic conditions. It is highly probable that in this experiment, the preponderance of bacteria growing under anaerobic conditions were faculative anaerobes.

Numbers of soil fungi were estimated by dilution plate counts on Rose Bengal-Streptomycin agar. The weaknesses of this technique are even more severe for soil fungi than bacteria because of the tendency of this technique to overestimate the profusely sporulating fungal species while drastically underestimating the sterile or slower growing species.

Data for the number of fungi in the Ottokee sand are shown in Figure 17, the Celina silt loam in Figure 18, and in the Paulding clay in Figure 19. The format for these Figures are the same as those discussed in the previous sections. The following general conclusions can be drawn:

1) The fungal population increased in size in response to sludge amendments. The direct relationship between the quantity of sludge and numbers of fungi is not as pronounced as with bacteria and actinomycetes.

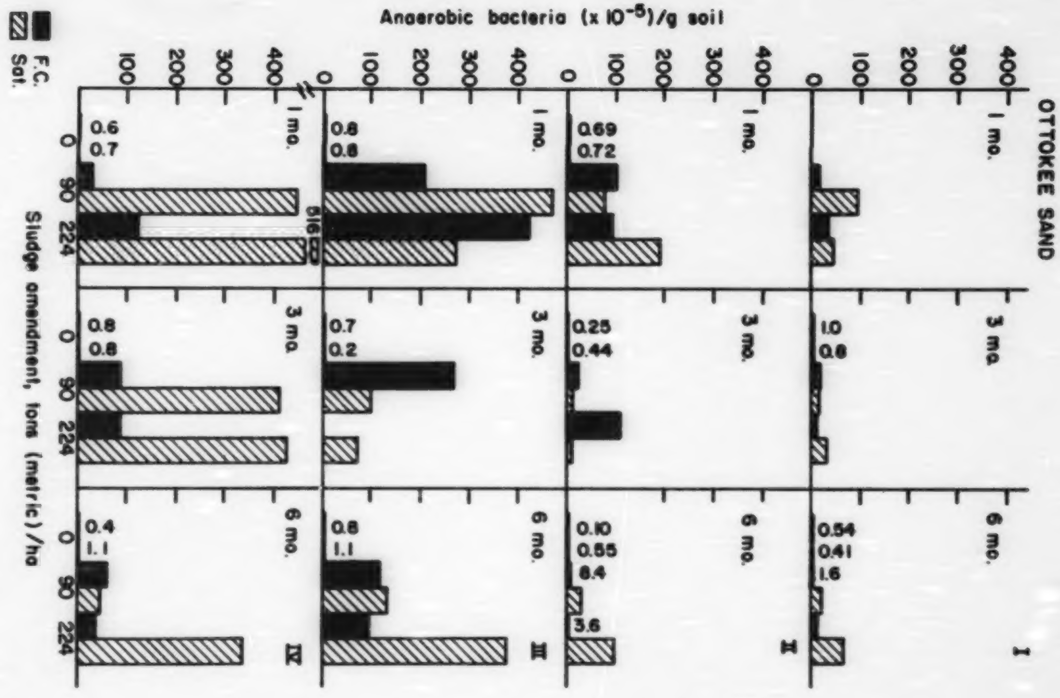

Figure 14. Dilution plate counts of anaerobic bacteria in sludge amended Ottokee sand as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV).

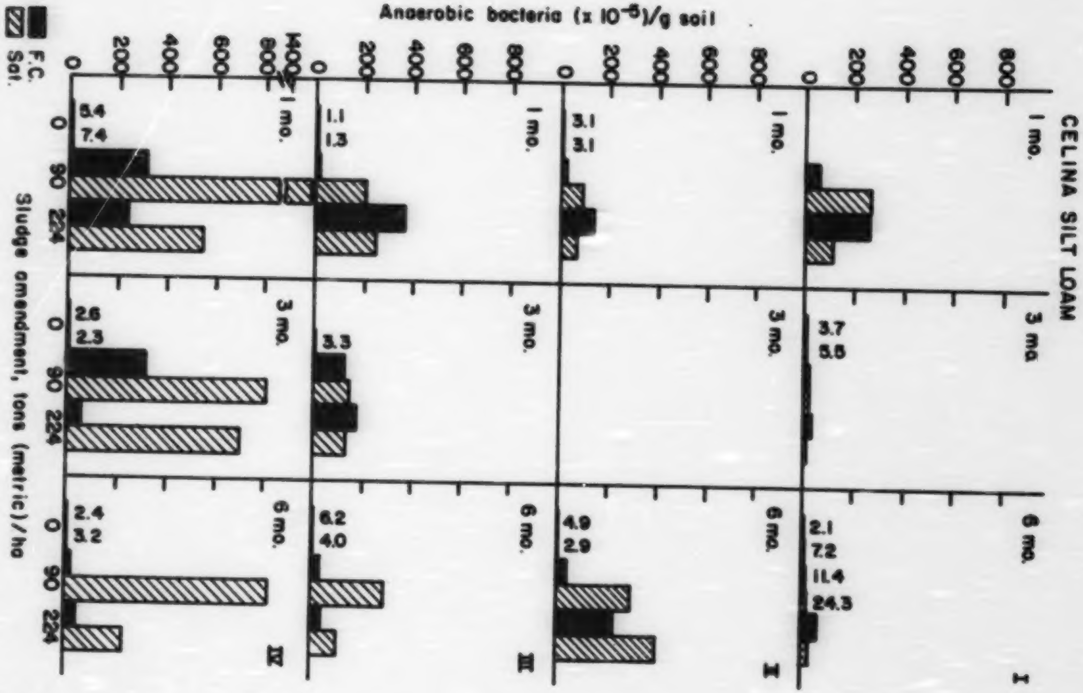

Figure 15. Dilution plate counts of anaerobic bacteria in sludge amended Celina silt loam as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV).

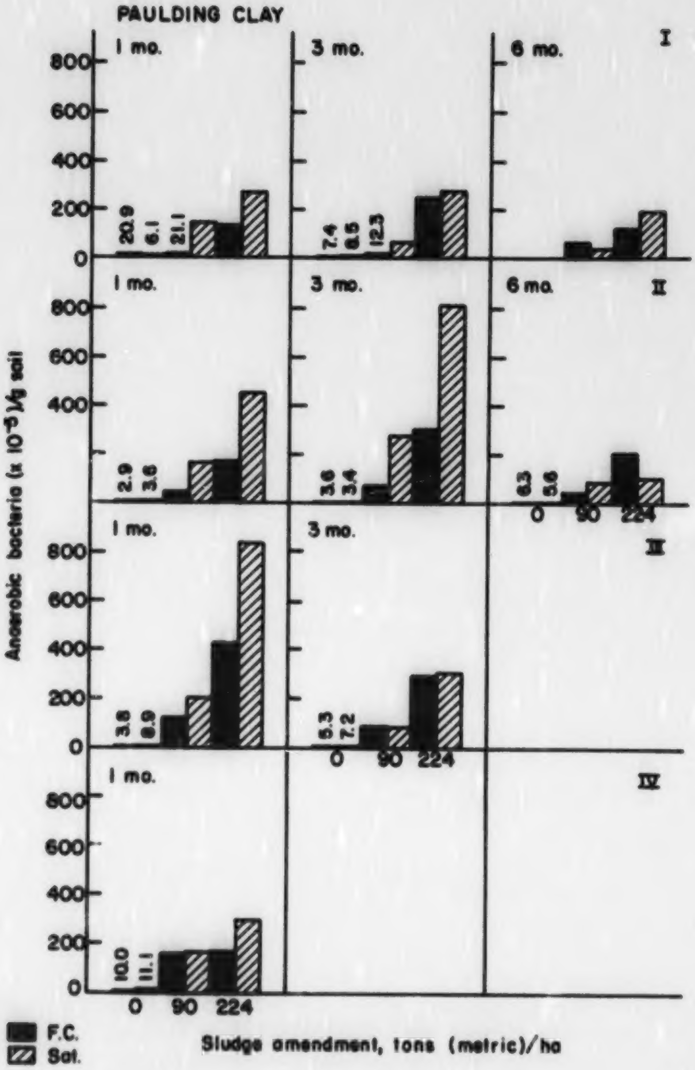

Figure 16. Dilution plate counts of anaerobic bacteria in sludge amended Paulding clay as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV).

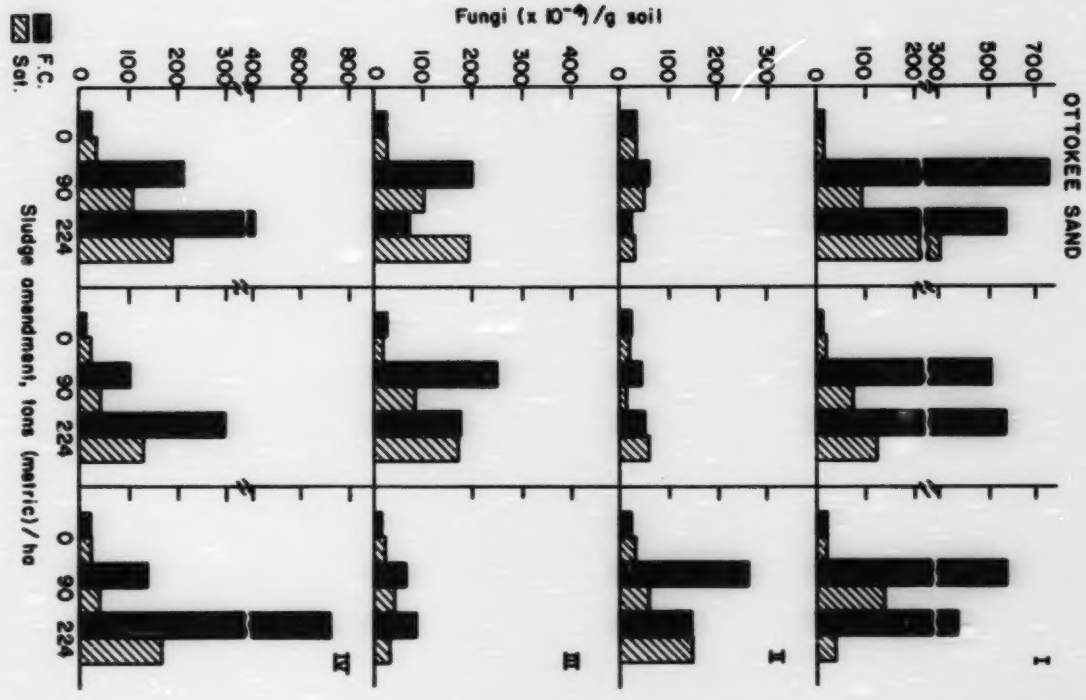

Figure 17. Dilution plate counts of soil fungi in sludge amended Ottokee sand as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV).

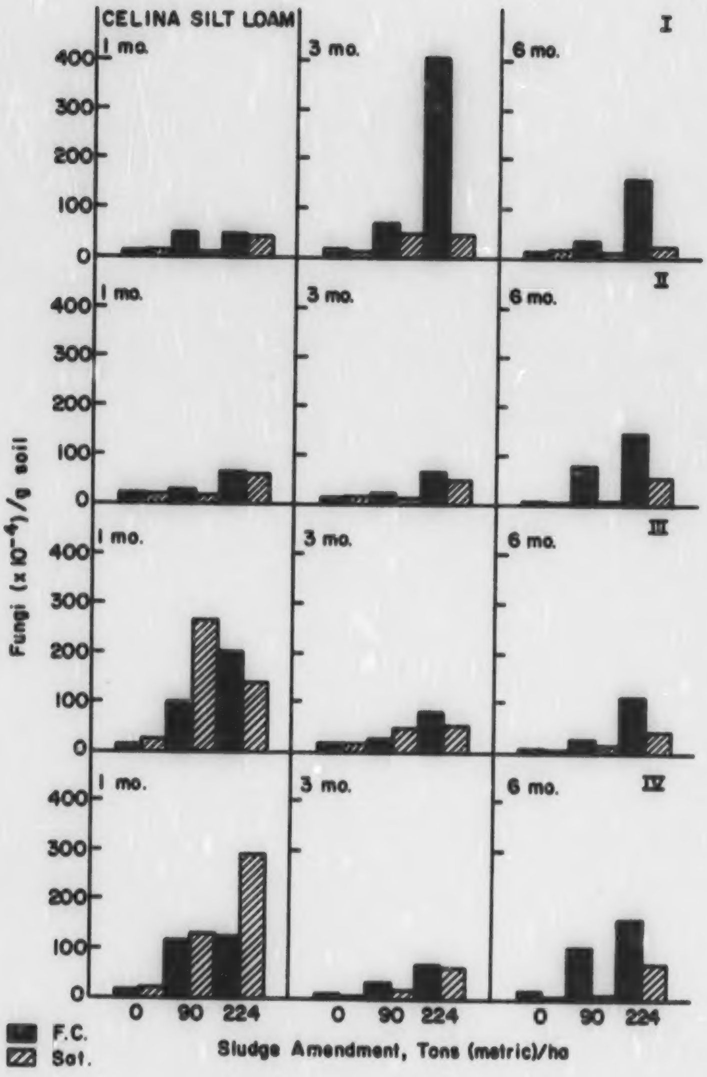

Figure 18. Dilution plate counts of soil fungi in sludge amended Celina silt loam as influenced by loading rate, soil moisture content, time of incubation, and temperature of incubation (Experiments I-IV).

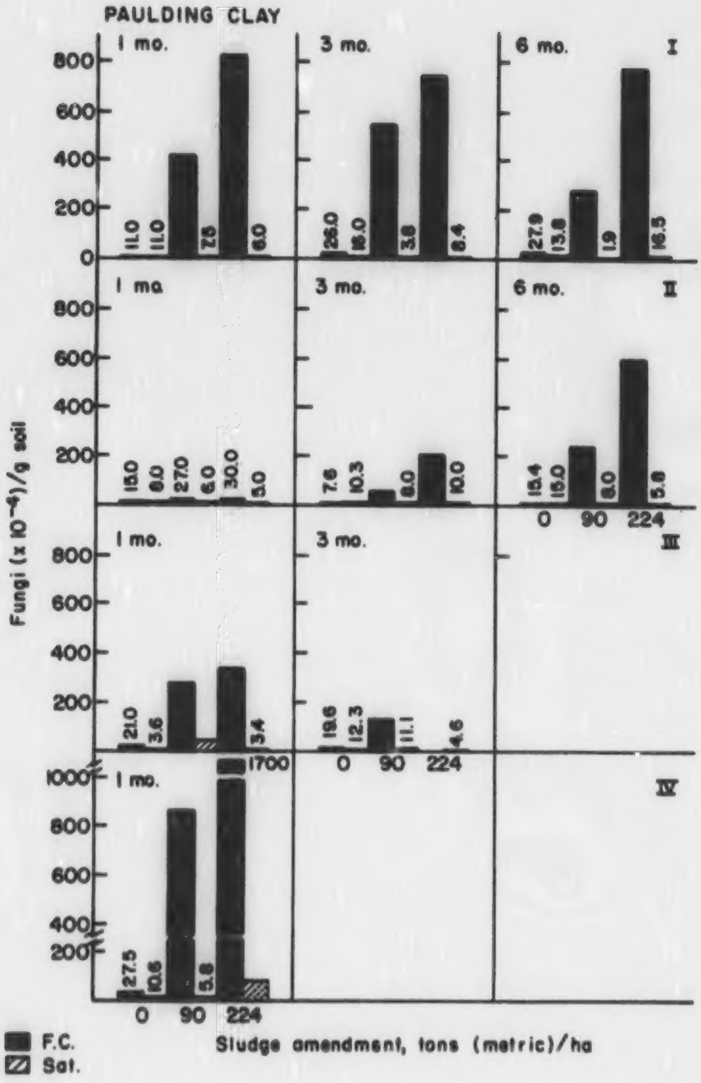

Figure 19. Dilution plate counts of soil fungi in sludge amended Paulding clay as influenced by loading rate, soil moisture content, time of incubation and temperature of incubation (Experiments I-IV).

2) The number of fungi did not decrease substantially with time of incubation as did the bacterial population. This might be expected since numerous fungal species would sporulate during the initial period of incubation and these spores would remain viable through the three and six month incubation period.

3) Numbers of fungi were equal to or less than the control soils in the sludge amended Paulding soil incubated at saturated moisture conditions. This development of anaerobic conditions in the saturated Paulding soil would eliminate fungi which are predominately aerobic.

4) In the Ottokee sand the largest numbers of fungi were also found at field capacity. In this soil we find an inverse relationship between the number of fungi and that of bacteria and actinomycetes at both field capacity and saturation (See Figure 11). This same relationship was not evident in either of the other two soils.

5) Numbers of fungi were less in the Celina silt loam incubated at field capacity than in the other two soils. The population difference between the soil incubated at field capacity and saturated conditions was not as extreme, however.

A comparison of population changes of fungi and bacteria in response to sludge amendments would indicate that both groups of organisms can participate in sludge decomposition. Fungi are probably dominant in the decomposition processes in the Ottokee sand at field capacity, while bacteria are dominant under saturated moisture conditions. In the Paulding clay both populations would seem to contribute significantly at field capacity, but the small amount of sludge decomposition under saturated conditions is all bacterial. In the Celina silt loam, bacteria probably dominate but fungi make a significant contribution. Data on numbers of anaerobic bacteria are nonconclusive and the significance of this group of microorganisms in sludge decomposition cannot be evaluated.

Survival of Indicator Bacteria in Soil

Survival of pathogenic microorganisms in soils which are used for disposal of waste effluents and sludges presents a potential health hazard which could limit the applicability and success of this approach (Miller, 1973). Surviving pathogens would provide a continuing risk to ground and surface water supplies, possibly contaminate vegetation in the sludge amended site, and present a health hazard to animals and humans in contact with the contaminated soils or vegetation.

In this study the Membrane Filter technique (American Public Health Assoc. et. al., Standard Methods, 1971) was used to evaluate the survival of total coliforms, fecal coliforms, and fecal streptococci from the anaerobically digested sewage sludge incorporated into soil. Data from these experiments are summarized in Table 10. Only a relative evaluation of the survival of these indicator bacteria are provided in Table 10 because variability between duplicate columns at each sampling period was often very high. This high

Table 10. RELATIVE SURVIVAL[a] OF INDICATOR BACTERIA IN SOILS AMENDED WITH ANAEROBICALLY DIGESTED SEWAGE SLUDGE.

Soil and Treatment	Total Coliforms			Fecal Coliforms			Fecal Streptococci		
	1 mo.	3 mo.	6 mo.	1 mo.	3 mo.	6 mo.	1 mo.	3 mo.	6 mo.
Ottokee									
90 Ton-FC	++	+	±	++	-	±	++	+	±
224 Ton-FC	+++	++	+	+++	-	-	++	++	±
90 Ton-Sat.	+++	++	+	++	-	-	++	±	±
224 Ton Sat.	++++	+++	±	++	-	-	+++	+	±
Celina									
90 Ton-FC	+++	+	±	+	-	-	+	±	-
224 Ton-FC	++++	+	±	+	-	-	++	++	+
90 Ton-Sat.	+++	++	+	++	-	-	++	±	-
224 Ton Sat.	+++	++	+	++	±	±	++	+	-
Paulding									
90 Ton-FC	++++	±	-	+++	-	-	+++	++	+
224 Ton-FC	++++	+	-	+++	-	-	++++	+++	++
90 Ton-Sat.	++++	±	-	++	-	-	+++	++	+
224 Ton-Sat.	++++	+	-	+++	-	-	++++	+++	++

[a] The relative comparisons above were based on the mean number of surviving indicator bacteria after the indicated incubation time over all four experiments. The initial population of indicator bacteria added with the sludge per gram of dry soil at the high and low loading rates was as follows: Total coliforms, 1.1×10^5 and 4.6×10^4; fecal coliforms, 1.2×10^3 and 4.5×10^2 ; fecal streptococci, 3.9×10^4 and 1.6×10^4.

Relative survival % = ++++, 30-40%; +++, 10-30%; ++, 1-10%; +, $< 1.0\%$; ±, $< 0.1\%$; -, No bacteria detected.

variability was associated primarily with the difficulty in predicting and selecting what soil serial dilutions would provide the proper number of colonies per filter. Over crowding or dilution to extinction were two common problems which occurred. A third problem was the interference of clay and silt sized particles in the development of characteristic colonies. It is the feeling of this investigator, however, that with greater experience and more adequate replication, this technique can provide quantitative data on survival of indicator bacteria.

A number of conclusions on the survival of indicator bacteria in the three experimental soils can be drawn from the data in Table 10.

1) The population of all surviving indicator bacteria after 1 months incubation never exceeded 40 per cent of that originally added with the sludge and continued to decrease with time.
2) In general, the percent survival of indicator bacteria was higher in the Paulding clay than in the other two coarser textured soils. This was particularly true for the fecal streptococci.
3) There was a trend for longer survival associated with soils incubated under saturated moisture conditions.
4) The fecal coliforms, perhaps because of their lower initial population,were almost completely eliminated from the soil by 3 month's incubation. Total coliforms and fecal streptococci were often detected in low numbers after 6 month's incubation.

Characterization of Bacterial Isolates

Aerobic heterotrophic bacteria and actinomycetes were isolated from dilution plates of sludge amended and control soil columns of all three experimental soils after 1 month's incubation. Isolations were made from soil columns incubated at each of the temperature series employed in the study (Experiments I-IV). The techniques employed in the isolation and maintenance of the isolates are described in the Materials and Methods.

A total of 354 bacterial isolates from the Ottokee sand and Celina silt loam soils and an additional 67 isolates from 0 day control and sludge amended treatments for these same soils were characterized extensively using the morphological, cultural and biochemical characters listed in Table 6. Characterization of bacterial isolates from the Ottokee sand and Celina silt loam soils (Experiments III & IV) and the Paulding clay soil was limited to morphological characters because of time limitations. In addition, none of the actinomycete isolates have been characterized in detail at the present time because of the inapplicability of many of the biochemical tests for these organisms.

The total number of original isolates, the initial survival, subsequent death and final number of bacterial isolates characterized are recorded in Table 11. The relatively low number of actinomycetes isolated does not indicate that they are less significant than the true bacteria in

Table 11. DISTRIBUTION OF BACTERIAL ISOLATES OBTAINED FROM DILUTION PLATING

	Ottokee Sand						Celina Silt Loam						
	Field Capacity			Saturated			Field Capacity			Saturated			
Observation	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Totals
Total No. of Isolates	35	40	31	36	40	40	40	48	61	53	58	57	549
Bacteria	30	38	31	33	40	40	35	56	59	45	56	51	517
Actinomycetes	5	2	0	3	0	0	5	2	2	8	2	3	32
Yeasts	0	0	0	0	0	0	0	0	0	0	0	2	2
% Loss at Isolation	20.0	27.5	25.8	36.0	47.5	12.5	30.0	10.3	24.6	18.9	34.5	14.0	Mean =25.1%
% Subsequent Loss	25.7	5.0	9.7	11.1	5.0	2.5	15.0	6.9	9.8	26.4	8.6	8.8	Mean =11.2%
Final No. of Bacterial Isolates Characterized	19	27	20	19	19	34	22	48	40	29	33	44	354

the decomposition of anaerobically digested sewage sludge in soil. Rather, the numbers are low because isolations were made from plates at the highest dilutions, which discriminated against actinomycetes which are less abundant than the true bacteria. The same argument can be used to explain the low incidence of yeasts among the isolates.

A mean value of 25.1 percent of the original isolates did not survive the initial transfer and an additional 11.2 percent died in subsequent transfers during the characterization period. Large but inconsistent differences in survival were evident in the isolates from the various soils and soil treatments (range of 10.3-47.5 percent loss). In general, the initial lack of growth was more prevalent in the isolates from the Ottokee sand, but death during subsequent transfers was higher in isolates from the Celina silt loam. A slight trend toward a higher percentage of survival of the isolates from sludge amended soils was also observed. The reason for poor survival by some of the isolates has not been ascertained. Possible reasons might include the choice of nutrient agar as the maintenance medium or the choice of incubation and storage temperatures.

The characterization data for the bacterial isolates are shown in the following tables. For convenience the results are listed in the following subgroupings: Morphological and cultural characteristics of the completely characterized isolated (Table 12) and morphological characteristics of others (Tables 13 and 14); growth characteristics (Table 15); biochemical and enzymatic activity (Table 16); acid production from selected carbohydrates, aerobic (Table 17); acid production from selected carbohydrates, anaerobic (Table 18); and antibiotic sensitivity (Table 19). The more significant observations gleaned from these data tables will be discussed in the following paragraphs. A more complete evaluation will require the development of similarity matrices for the isolates using a system of Numerical Taxonomy.

The morphological and cultural characteristics of the bacterial isolates from the isolates which were characterized in detail are summarized in Table 12. It is important to note that most generalizations which follow in this and other paragraphs are consistent except for the Ottokee sand treatments incubated at field capacity. Probably insufficient water was present at field capacity in this coarse textured soil to provide water films around soil particles as sites for bacterial proliferation. Reference to Figure 11 supports this contention by showing that the bacterial populations increased very little in sludge amended Ottokee sand incubated at field capacity.

Over 90 percent of the isolates from both soils and in both the control and sludge amended soils were rod or coccobacillary shaped cells. Pleomorphic cells, curved rods, and cocci forms made up a small percentage of the isolates and were found almost exclusively in the sludge amended Celina soil. About 75 percent of the bacteria from sludge amended soils occurred as single cells in culture, while those from the unamended soils had a greater tendency to form chains of four or more cells. Of considerable interest was the marked change of the bacterial population from one dominated by gram positive bacteria in the unamended soil to one where gram negative bacteria constituted about 50 percent or greater of the population.

Table 12. MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIAL ISOLATES (EXPERIMENTS I & II)

	Ottokee Sand						Celina Silt Loam					
	Field Capacity			Saturated			Field Capacity			Saturated		
Character	Con-trol	90 Ton	224 Ton	Con-rol	90 Ton	224 Ton	Con-trol	90 Ton	224 Ton	Con-rol	90 Ton	224 Ton
	% of Isolates Positive											
Rods	68.4	70.4	85.0	78.9	84.2	76.5	86.4	83.3	57.5	93.1	87.9	88.6
Curved Rods	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	3.0	0.0
Cocco-bacillary	26.3	25.9	15.0	21.1	15.8	23.5	13.6	16.7	35.0	6.9	9.1	9.1
Cocci	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	2.3
Spores	5.3	3.7	0.0	10.5	5.3	2.9	18.2	2.1	7.5	13.8	6.1	2.3
Pleomorphic	5.3	0.0	0.0	0.0	0.0	0.0	0.0	6.3	2.5	0.0	3.0	0.0
Singles	57.9	63.0	60.0	57.9	68.4	85.3	45.5	79.2	75.0	44.8	75.8	79.5
Chains > 4 cells	31.6	37.0	40.0	42.1	21.1	14.7	50.0	20.8	22.5	55.2	12.1	20.5
Length-0.2-0.6 µm	5.3	3.7	5.0	10.5	15.8	5.9	4.5	14.6	20.0	10.3	12.1	15.9
" 0.6-1.2 µm	57.9	70.4	75.0	52.6	73.7	70.6	50.0	56.3	57.5	41.4	63.6	52.3
"> 1.2 µm	31.6	25.9	20.0	36.8	10.5	23.5	40.9	27.1	17.5	48.3	24.2	31.8
Width-<0.5 µm	5.3	11.1	10.0	21.1	47.4	38.2	9.1	27.1	27.5	27.6	39.4	27.3
" 0.5-1.0 µm	68.4	77.8	90.0	68.4	47.4	50.0	72.7	72.9	70.0	51.7	54.4	63.6
">1.0 µm	21.1	11.1	0.0	10.5	5.3	11.8	18.2	0.0	2.5	20.7	6.1	9.1
Gram Negative	31.6	25.9	40.0	36.8	68.4	67.6	22.7	43.8	45.0	31.0	69.7	52.3
" Positive	63.2	74.1	60.0	63.2	31.6	32.4	77.3	54.2	55.0	69.0	30.3	47.7
" Variable	5.3	0.0	0.0	15.8	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0
Mobility	89.5	81.5	90.0	78.9	73.7	81.8	81.8	93.8	85.0	89.7	90.9	79.5
Colony White	78.9	63.0	55.0	73.7	68.4	55.9	81.8	64.6	70.0	79.3	69.7	56.8
" Yellow	21.1	29.6	35.0	26.3	31.6	26.5	18.2	31.3	15.0	17.2	21.2	34.1
" Pink	0.0	0.0	0.0	0.0	0.0	2.9	4.5	2.1	2.5	0.0	0.0	0.0
" Orange	0.0	7.4	5.0	0.0	0.0	5.9	0.0	0.0	12.5	3.4	0.0	2.3
" Purple	0.0	0.0	10.0	0.0	0.0	5.9	0.0	2.1	0.0	0.0	9.1	6.8

Table 13. MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL ISOLATES FROM THE OTTOKEE SAND AND CELINA SILT LOAM SOILS (EXPERIMENTS III & IV).

	Ottokee Sand						Celina Silt Loam					
	Field Capacity			Saturated			Field Capacity			Saturated		
Character	Con-trol	90 Ton	224 Ton	Con-trol	90 Ton	224 Ton	Con-trol	90 Ton	224 Ton	Con-trol	90 Ton	224 Ton
	% of Isolates Positive											
Rods	91.3	93.8	65.6	80.0	80.8	93.5	78.3	86.7	71.4	86.7	93.8	83.3
Curved Rods	0.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0	0.0	0.0	0.0	0.0
Cocco-bacillary	8.7	6.3	34.4	20.0	19.2	9.7	34.8	13.3	26.7	13.3	6.3	16.7
Spores	4.3	6.3	6.3	12.0	7.7	6.5	17.4	13.3	14.3	6.7	6.3	0.0
Singles	69.6	84.4	90.6	92.0	88.5	93.5	82.6	60.0	85.7	80.0	87.5	94.4
Chains												
>4 cells	34.4	15.6	9.4	8.0	11.5	6.5	21.1	40.0	14.3	20.0	6.3	5.6
Length												
0.2-0.6 µm	17.4	0.0	3.1	8.0	7.7	3.2	8.7	6.7	0.0	0.0	12.5	0.0
" 0.6-1.2 µm	60.9	84.4	78.1	68.0	80.8	83.9	73.9	46.7	78.6	66.7	81.3	88.8
"> 1.2µm	21.7	15.6	12.5	24.0	11.5	12.9	34.8	46.7	21.4	33.3	6.3	16.7
Width												
> 0.5 µm	21.7	21.9	28.1	20.0	46.1	48.4	13.0	13.3	28.6	13.3	31.3	22.2
" 0.5-1.0 µm	65.2	62.5	62.5	72.0	50.0	41.9	95.7	46.7	57.1	73.3	68.8	77.8
" > 1.0 µm	13.0	12.5	6.3	8.0	3.8	6.5	8.7	40.0	14.3	13.3	0.0	0.0
Gram												
Negative	43.5	50.0	34.4	40.0	57.7	64.5	17.4	20.0	35.7	26.7	56.3	50.0
" Positive	56.5	46.9	65.6	56.0	42.3	35.5	82.6	80.0	64.3	73.3	43.8	50.0
No. of isolates	23	32	32	25	26	31	23	15	14	15	16	18

Table 14. MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL ISOLATES FROM THE PAULDING CLAY SOILS (EXPERIMENTS I - IV).

	Paulding Clay					
	Field Capacity			Saturated		
Character	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
	% of Isolates Positive					
Rods	100.0	100.0	88.2	78.6	80.0	66.7
Cocco-bacillary	0.0	0.0	11.8	21.4	20.0	40.0
Spores	8.3	0.0	5.9	7.1	6.7	0.0
Singles	66.7	69.2	82.4	71.4	93.3	66.7
Diplo Form	0.0	0.0	5.9	0.0	6.7	6.7
Chains > 4 cells	33.3	30.8	11.8	28.6	0.0	20.0
Length						
0.2-0.6 µm	0.0	23.0	11.8	0.0	0.0	13.3
" 0.6-1.2 µm	66.7	38.5	64.7	64.3	93.3	60.0
" > 1.2 µm	33.3	38.5	23.5	35.7	6.7	26.7
Width						
> 0.5	0.0	38.5	11.8	7.1	20.0	0.0
" 0.5-1.0 µm	75.0	38.5	64.7	64.3	73.3	73.3
" > 1.0 µm	25.0	23.0	23.5	28.6	6.7	26.7
Gram						
Negative	25.0	46.2	35.3	7.1	80.0	73.3
Positive	75.0	53.8	64.7	92.9	20.0	26.7
No. of isolates	12	13	17	14	15	15

Table 15. GROWTH CHARACTERISTICS OF BACTERIAL ISOLATES

	Ottokee Sand						Celina Silt Loam					
	Field Capacity			Saturated			Field Capacity			Saturated		
Character	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
						% of Isolates Positive						
Relative												
Growth[a] - 1	77.8	65.4	80.0	78.9	63.2	33.3	95.5	64.6	40.0	63.0	41.9	44.8
" 3	11.1	19.2	10.0	10.5	26.3	25.0	4.5	20.8	53.3	29.6	38.7	24.1
" 5	11.1	15.4	10.0	10.5	10.5	37.5	0.0	12.5	6.7	3.7	12.9	31.0
Even Turbidity	6.3	19.0	5.3	10.5	21.1	29.4	11.8	10.4	20.0	10.3	40.6	29.5
Flocculent Turbidity	6.3	0.0	0.0	0.0	0.0	0.0	5.9	0.0	5.0	0.0	5.0	0.0
Sediment	87.5	71.4	57.9	89.5	73.7	58.8	82.4	85.4	67.5	75.9	37.5	52.3
Pellicle	0.0	9.5	26.3	0.0	5.3	11.8	0.0	4.2	7.5	10.3	15.6	18.2
Growth at												
" 5°C	42.1	63.0	70.0	47.4	57.9	76.5	59.1	68.8	55.0	24.7	51.5	52.3
" 20°C	89.5	96.3	100.0	94.7	100.0	94.1	100.0	95.5	95.0	93.1	100.0	97.7
" 35°C	68.4	70.4	45.0	89.5	68.4	67.6	81.8	77.1	77.5	82.8	69.7	72.7
Growth in												
NaCl 2.5%	94.7	96.3	95.0	94.7	73.4	88.2	72.7	93.8	97.5	86.2	75.8	93.2
" 7.5%	36.8	40.7	25.0	15.8	21.1	26.5	27.3	45.8	60.0	24.1	33.3	27.3
" 12.5%	5.3	7.4	5.0	0.0	5.3	5.9	4.5	6.3	12.5	0.0	9.1	2.3

[a] Maximum growth in solution culture occurred after 1, 3 and 5 days of incubation.

Table 16. BIOCHEMICAL AND ENZYMATIC ACTIVITY OF BACTERIAL ISOLATES

	Ottokee Sand						Celina Silt Loam					
	Field Capacity			Saturated			Field Capacity			Saturated		
Character	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
	% of Isolates Positive											
Hydrolysis of:												
Starch	54.5	20.8	15.0	31.0	27.3	15.9	31.6	22.2	0.0	42.1	10.5	2.9
Cellulose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pectin	4.5	5.0	12.5	6.9	6.1	0.0	5.3	0.0	0.0	5.3	0.0	6.7
Gelatin	28.6	27.1	17.5	24.1	30.3	34.1	31.6	18.5	15.0	21.1	15.8	8.8
Trybutyrin	0.0	2.1	0.0	0.0	6.1	2.3	0.0	3.7	0.0	0.0	5.3	0.0
Triolein	9.1	8.3	7.5	10.3	9.1	2.3	0.0	0.0	0.0	15.8	0.0	20.6
Production of:												
Catalase	46.4	58.3	67.5	44.8	54.5	56.8	57.9	88.9	80.0	42.1	78.9	58.8
Cytochrome Oxidase	27.3	31.3	47.5	27.6	63.6	43.2	26.3	18.5	55.0	31.6	36.8	50.0
Urease	22.7	14.6	17.5	13.8	9.1	11.4	15.8	13.3	15.0	26.3	26.3	11.8
Indole	0.0	0.0	2.6	3.6	3.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0
Acetylmethyl-Carbinol	0.0	0.0	5.1	0.0	0.0	0.0	5.3	3.7	0.0	0.0	5.3	0.0
Acid (Methyl red)	13.6	0.0	7.7	10.3	3.0	11.4	5.6	3.7	0.0	10.5	5.6	0.0
H_2S	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	3.1
Reduction of:												
Nitrate (+gas)	9.1	6.3	7.7	6.9	9.1	11.4	5.3	11.1	10.0	5.3	5.3	3.1
Nitrate (no gas)	40.9	37.5	33.3	48.3	45.5	38.6	0.0	40.7	25.0	21.1	42.1	43.8
Utilization of												
Citrate	13.6	16.0	12.5	10.3	39.4	25.0	5.3	37.0	25.0	26.3	47.4	51.5
Litmus Milk												
Peptonized												
Total	13.6	22.9	10.0	13.8	33.3	15.9	26.3	7.4	5.0	10.5	15.8	8.8
Surface	45.5	33.3	32.5	51.7	33.3	36.4	21.1	55.6	30.0	26.3	15.8	23.5
Acid	3.9	18.2	14.6	10.0	17.2	15.2	21.1	3.7	0.0	21.1	10.5	10.5
Colorless	22.7	0.0	10.0	3.4	9.1	2.3	10.5	22.2	10.0	5.3	0.0	5.9
Alkaline	36.4	45.8	32.5	48.3	51.5	45.5	10.5	48.1	40.0	26.3	47.4	47.1
Acid > Alkal.	22.7	35.4	30.0	27.6	27.3	34.1	21.1	25.9	30.0	36.8	26.3	11.8
Alkal. > Acid	13.6	20.8	12.5	34.5	27.3	20.5	5.3	3.7	10.0	0.0	15.8	17.6

Table 17. ACID PRODUCTION FROM SELECTED CARBOHYDRATES (AEROBIC).

	Ottokee Sand						Celina Silt Loam					
	Field Capacity			Saturated			Field Capacity			Saturated		
Carbohydrate	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
Glucose	36.8	25.9	5.0	10.5	21.1	8.8	22.7	10.4	17.5	17.2	12.1	27.3
Fructose	36.8	29.6	5.0	15.8	21.1	5.9	45.5	18.8	22.5	44.8	18.2	22.7
Mannose	36.8	33.3	5.0	10.5	21.1	5.9	36.4	16.7	22.5	41.4	15.2	22.7
Galactose	36.8	29.6	5.0	10.5	26.3	8.8	36.4	10.4	17.5	37.9	18.2	22.7
Arabinose	26.3	22.2	10.0	5.3	21.1	2.9	22.7	2.1	22.5	27.6	9.1	20.5
Lactose	21.1	18.5	0.0	5.3	15.8	2.9	13.6	10.4	12.5	13.8	9.1	18.5
Sucrose	26.3	11.1	5.0	5.3	10.5	5.9	40.9	14.6	22.5	44.8	15.2	18.2
Maltose	31.6	18.5	5.0	10.5	5.3	2.9	40.9	18.8	22.5	34.5	15.2	15.9
Cellobiose	36.8	25.9	5.0	5.3	15.8	2.9	31.8	22.9	20.0	20.7	12.1	20.5
Raffinose	36.8	14.8	5.0	10.5	10.5	5.9	18.2	6.3	17.5	24.1	6.1	15.9
Dextrin	26.3	22.2	5.0	5.3	10.5	5.9	36.4	37.5	40.0	48.3	15.2	18.2
Inulin	21.1	14.8	5.0	5.3	10.5	5.9	18.2	4.2	12.5	41.4	24.2	18.2
Mannitol	31.6	22.2	5.0	10.5	10.5	5.9	27.3	10.4	30.0	34.5	18.2	13.6
Sorbitol	26.3	18.5	5.0	5.3	15.8	5.9	13.6	4.2	25.0	20.7	9.1	15.9
Dulcitol	21.1	22.2	0.0	10.5	5.3	0.0	18.2	0.0	10.0	24.1	0.0	9.1
Ribose	5.3	0.0	0.0	5.3	5.3	0.0	21.1	3.4	15.4	17.6	6.3	0.0
	28.6	22.0	5.4	8.2	14.2	5.5	27.7	12.7	20.7	30.8	13.6	18.7

Table 18. ACID PRODUCTION FROM SELECTED CARBOHYDRATES (ANAEROBIC)

Carbon Sources	Ottokee Sand						Celina Silt Loam					
	Field Capacity			Saturated			Field Capacity			Saturated		
	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
	% of Isolates Positive											
Glucose	44.4	21.1	20.0	52.6	26.3	20.6	36.4	39.6	32.5	55.2	45.5	29.5
Fructose	48.1	21.1	15.0	31.6	5.3	5.9	50.0	22.9	15.0	31.0	45.5	25.5
Mannose	26.3	14.8	5.0	21.1	5.3	26.5	40.9	20.8	25.0	27.6	18.2	25.0
Galctose	15.8	14.8	20.0	42.1	15.8	11.8	36.4	14.6	25.0	24.1	24.2	18.2
Arabinose	15.8	3.7	5.0	10.5	0.0	0.0	13.6	2.1	12.5	3.4	9.1	11.4
Lactose	25.9	20.0	5.3	42.1	42.1	8.8	31.8	22.9	10.0	37.9	15.2	13.6
Sucrose	55.0	44.4	31.6	36.8	36.8	17.6	40.9	16.7	17.5	27.6	21.2	20.5
Maltose	30.0	15.8	7.4	36.8	26.3	11.8	36.4	8.3	10.0	24.1	15.2	25.0
Cellobiose	5.3	3.7	5.0	10.5	0.0	5.9	13.6	8.3	7.5	6.9	9.1	9.1
Inulin	5.3	9.6	4.3	5.3	5.3	17.6	27.3	8.3	12.5	20.7	15.2	9.1
Sorbitol	0.0	7.4	5.0	5.3	0.0	0.0	0.0	10.0	10.0	3.4	3.0	6.8
Dulcitol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0
Mean	36.6	14.7	9.3	26.8	20.4	14.1	32.7	15.5	15.0	23.8	20.1	17.6

Table 19. SENSITIVITY OF BACTERIAL ISOLATES TO SEVEN SELECTED ANTIBIOTICS

	Ottokee Sand						Celina Silt Loam					
	Field Capacity			Saturated			Field Capacity			Saturated		
Antibiotic	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
	% Sensitive Isolates											
Viomycin	63.2	70.4	50.0	68.4	47.4	44.1	63.6	45.8	55.0	69.0	45.5	34.1
Streptomycin	89.5	88.9	95.0	84.2	73.7	88.2	86.4	77.1	75.0	82.8	78.8	90.9
Chloromycetin	89.5	88.9	90.0	78.9	89.5	91.2	90.9	85.4	90.0	89.7	87.9	86.4
Novobiocin	94.7	77.8	75.0	63.2	63.2	58.8	95.5	72.9	70.0	79.3	60.6	61.4
Bacitracin	68.4	77.8	65.0	52.6	52.6	44.1	81.8	58.3	70.0	72.4	33.3	52.3
Tetracycline	100.0	96.3	90.0	94.7	100.0	100.0	100.0	97.9	95.0	86.2	97.0	93.2
Penicillin	68.4	63.0	70.0	52.6	42.1	38.2	72.7	52.1	55.0	55.2	42.4	36.4
Mean	81.3	80.4	76.4	70.7	66.9	66.4	84.4	69.9	72.9	76.4	63.6	65.0

This change and other changes discussed below reflects an alteration of the soil microbial environment. The functional significance of these changes is presently not apparent, but they are academically interesting. Accompanying the change in gram reaction for bacterial isolates from sludge amended soils was reduction in the number of spore formers, a reduction in overall cell size and an increase in the number of pigmented isolates. Most of the isolates were highly motile and did not differ greatly regardless of their origin. This last result was opposite from 0 day isolates which average only 29 per cent motile. Morphological data for the otherwise uncharacterized isolates from the Ottokee sand and Celina silt loam (Table 13) and the Paulding clay (Table 14) agree closely with the generalization above. In fact, the most dramatic change in population, based on gram reaction, occurred in the Paulding clay soil columns incubated under saturated conditions. Here the control population changed from 90 per cent gram positive bacteria to greater than 70 per cent gram negative bacteria in the sludge amended treatments.

Bacterial isolates from sludge amended soils also differed considerably in growth characteristics from the normal soil bacterial population (Table 15). Isolates from sludge amended soils usually grew at a faster relative growth rate, grew better at 5° C but less well at 35° C, tolerated higher concentrations of Na Cl, and exhibited a greater tendency for even turbidity or pellicle formation in broth culture. The development of a more salt tolerant population reflects the selection pressure associated with the high accumulation of soluble salts in sludge amended soils.

Data on the biochemical activities of the bacterial isolates are provided in Table 16. In general, the biochemical activities of the isolates did not show the consistent trend differences between treatments previously found with morphological and cultural characters. The exceptions to this conclusion were the increased ability of isolates from sludge amended soils to utilize citrate, to have increased catalase and cytochrome oxidase activity, and reduced hydrolytic activity on starch. The ability to reduce nitrate with and without production of N_2 differed little in the Celina soil isolates with and without sludge amendments, but was found more frequently in isolates from sludge amended Ottokee sand. Proteolytic activity was weaker in the bacterial isolates from sludge amended Ottokee sand (see data for gelatin hydrolysis and peptonization of litmus milk) but not in the Celina isolates. The biochemical tests frequently used for the characterization of enteric bacteria ie. production of acetylmethylcarbinol, acid (methyl red), indol, and H_2S, provided a very low percentage of positive responses with no apparent differences between soils or treatments. Lipid esterase, cellulase, and pectinase activity were either low or entirely absent among the isolates. The combination of responses possible on litmus milk were largely inconsistent.

The ability of the bacterial isolates to produce acid from a series of selected carbohydrates was evaluated under both aerobic and anaerobic conditions (Tables 17 and 18). Bacterial isolates from sludge amended soils were less able to produce acid from the various carbohydrates under aerobic conditions than were the isolates from the control soils. The overall activity of the isolates from the saturated Celina soil also surpassed that of the saturated Ottokee sand isolates. The same general trends were evident under anaerobic conditions but the overall number of positive cultures from the Ottokee sand increased considerably. Antibiotic

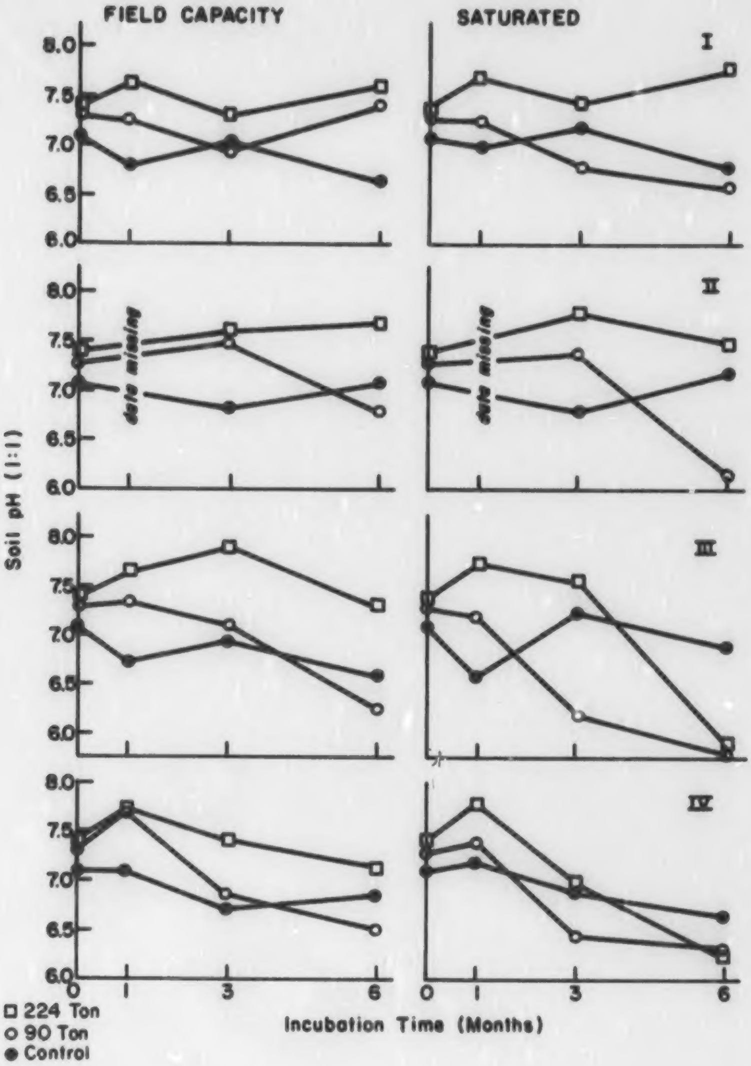

Figure 20. Soil pH in incubated columns of sludge amended and control Ottokee sand (Experiments I-IV).

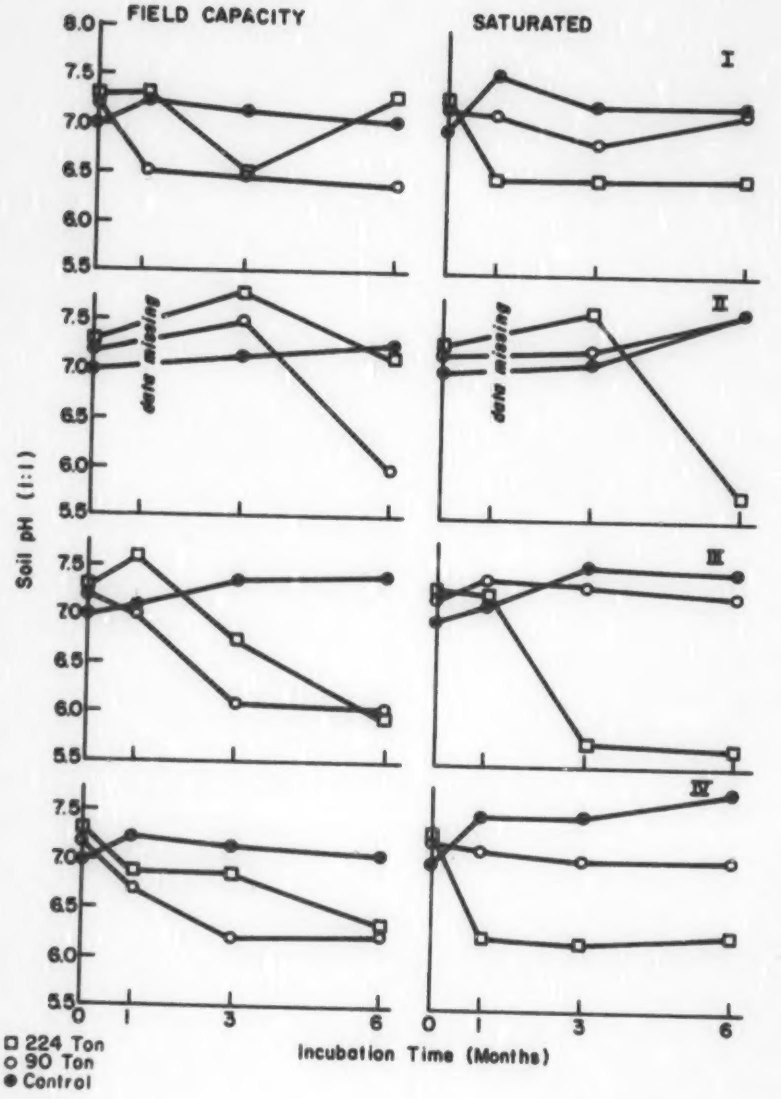

Figure 21. Soil pH in incubated columns of sludge amended and control Celina silt loam (Experiments I-IV).

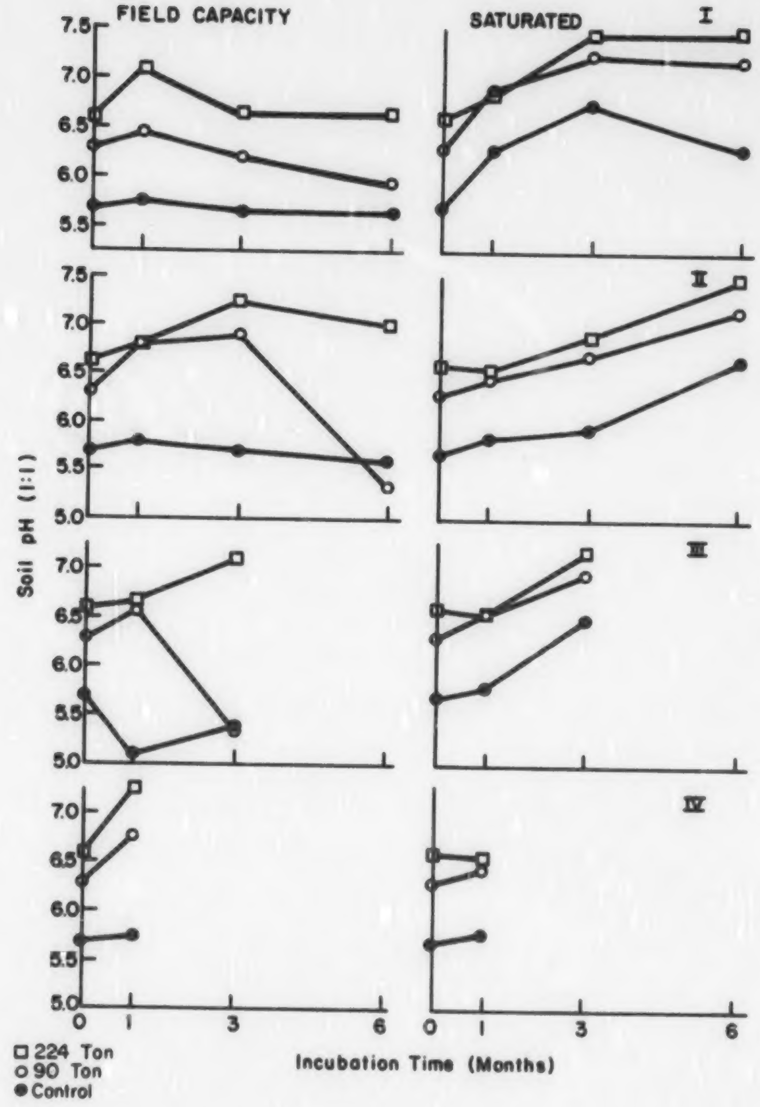

Figure 22. Soil pH in incubated columns of sludge amended and control Paulding clay (Experiments I-IV).

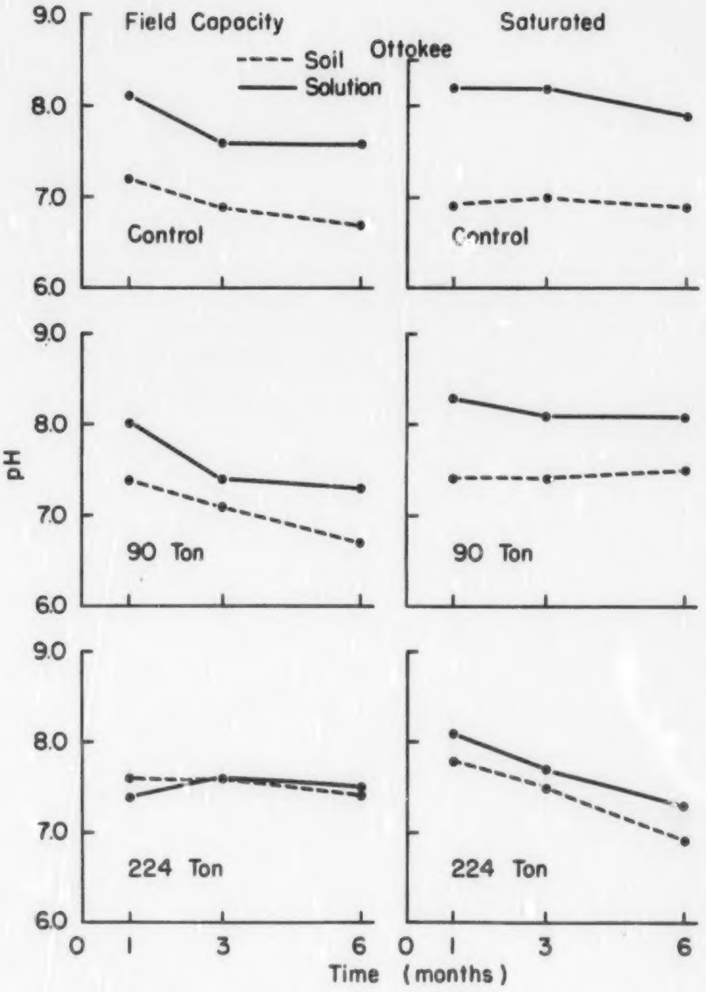

Figure 23. Relationship of solution pH to soil pH (Ottokee sand).

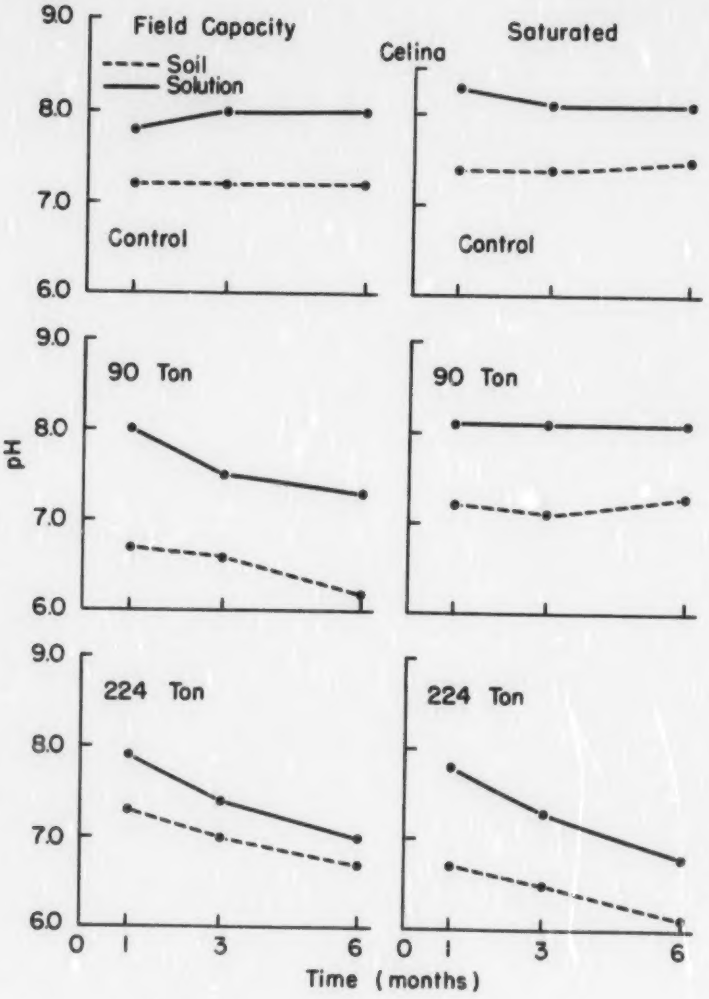

Figure 24. Relationship of solution pH to soil pH (Celina silt loam)

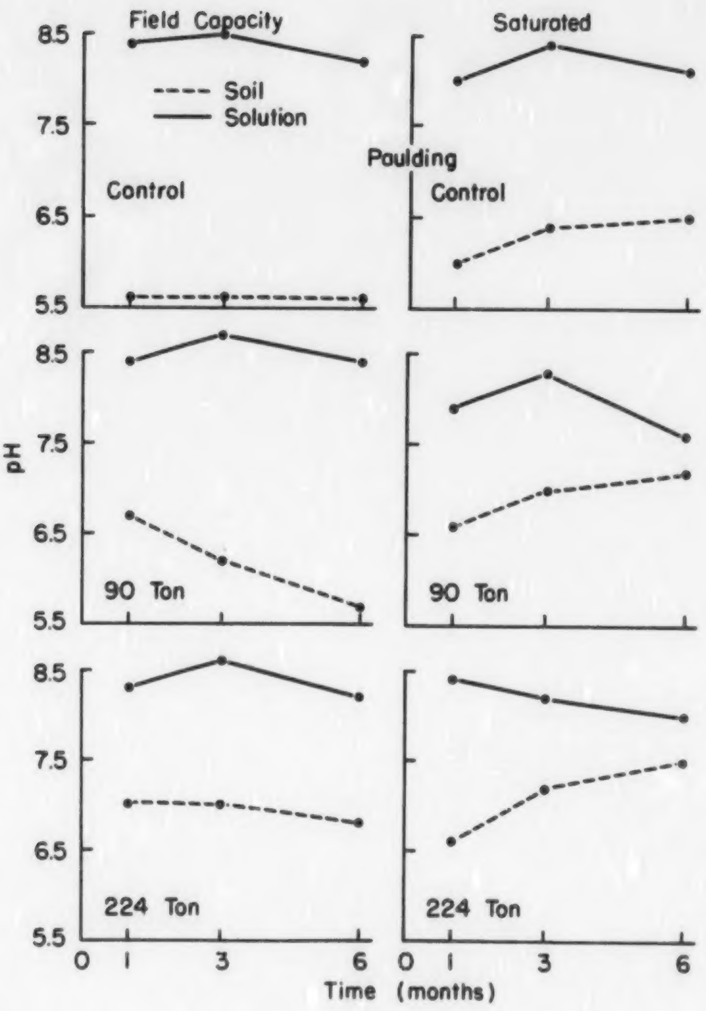

Figure 25. Relationship of solution pH to soil pH (Paulding clay).

sensitivity was the last group of characters utilized for the evaluation of the bacterial isolates. The data shown in Table 19 shows a general increase in resistance to four of the seven antibiotics. The exceptions were streptomycin, chloromycetin and tetracycline. As was the case with a number of other characters, the isolates from the sludge amended Ottokee sand treatments incubated at field capacity differed little in antibiotic sensitivity from the control.

PHASE 3: ANALYSES OF THE DISPLACED SOIL SOLUTIONS

Analyses of the displaced soil solutions from the various sludge amended columns provide an indirect evaluation of microbial activity. The presence or absence of various inorganic or organic compounds or ions in the soil solution eg. soluble salts, nitrogen compounds, heavy metal ions, etc. are often directly or indirectly related to microbial activity. The presence of these soluble materials is of considerable importance to the utility of sludge disposal on soil, since excessively large accumulations of some ions or organic compounds could affect ground water quality if movement through the profile would occur or be phytotoxic to the associated vegetative cover. Since these studies were based on laboratory incubation experiments, the concentration of soluble constituents found may not be directly applicable to field conditions. Under natural conditions both plant uptake and leaching could be expected to decrease the concentration of soluble compounds found at any one time in the soil plow layer. However, it should be pointed out that the concentrations of any one soluble constituent found in this study will reflect the total supplying power of the incorporated sewage sludge for this constituent during a particular set of experimental conditions.

Soil and Solution pH

Hydrogen ion activity in the sludge amended soil is of considerable significance because of the impact of pH on both microbial activity and the solubility and availability of many nutrient and heavy metal cations. Data showing changes in the soil pH with time under different temperature of incubation are shown in Figures 20, 21, 22. In general, the addition of anaerobically digested sewage sludge raised the initial pH of all three soils. This was followed by a subsequent drop in pH in all soil treatments where appreciable nitrification of sludge nitrogen occurred. (See Figures 26-43). The magnitude of this pH drop was almost always directly related to the amount of nitrate formed. The pH of sludge amended soils remained above the control soils in those treatments where anaerobic conditions decreased nitrification or accelerated biological denitrification, or where an apparent sludge toxicity decreased the activity of the nitrifying bacteria. These instances will be noted specifically in the subsequent discussion of soluble nitrogen.

What does seem readily apparent is that the addition of sewage sludge to soils maintained under proper moisture conditions will result in an appreciable decrease in soil pH. This drop in pH could alter the solubility of various metal cations, that this actually occurred can be seen from the plant analysis data shown in Figures 49 & 50 for Cu and Zn and perhaps for B (Figure 52).

The relationship of the pH of the displaced soil solution and the soil pH is shown in Figures 23, 24, 25. In general, the solution pH was considerably higher than that of the soil from which it was displaced, the magnitude of the difference being greater in the finer textured Paulding and Celina soils. These differences can be explained as an example of the suspension effect (McLean and Franklin, 1964) which increases the activity of cations near the surface of the soil colloids (in this case H^+) compared to the displaced soil solution. The observations that the difference in hydrogen ion activity between the soil and displaced soil solution decreases as the electrolyte concentration increases eg. higher rates of sludge amendment, or when the soils are incubated at field capacity rather than under saturated moisture conditions, are also in line with the idea of a suspension effect (Franklin and McLean, 1963).

Specific Conductance

The accumulation of excess soluble salts after application of sewage sludge to soil is another potential problem which might affect the utility of land disposal of sludges (Hinesly et.al. 1972). Plant growth and seed germination are both adversely affected by soluble salt accumulation, although plant species differ greatly in their tolerance (Richards, 1954). The mechanisms responsible for reduced growth include the reduced availability of soil water (osmotic effects), toxicity of certain of the soluble ions, and the induction of nutrient imbalances because of excess salts.

Specific conductance of the displaced soil solutions from sludge amended and control soils was measured to evaluate the possible severity of salt accumulations. These data are shown in Tables 20, 21, 22. The relationship of specific conductivity to a potential crop response can be estimated from the response table for saturation extracts shown below (Table 23).

Table 23. RELATIONSHIP OF CROP RESPONSE TO SOIL SALINITY EXPRESSED IN TERMS OF THE CONDUCTIVITY OF THE SATURATION EXTRACT. (RICHARDS, 1954)

Saline effects mostly negligible	Yields of very sensitive crops may be restricted	Yields of many crops restricted	Only tolerant crops yield satisfactorily	Only a few very tolerant crops yield satisfactorily

0 2 4 8 16

Scale of conductivity (mmhos/cm at 25°C)

Specific conductivity of displaced soil solutions from soils incubated under saturated soil conditions may be compared directly with Table 23. Specific conductivity of solutions from soils incubated at field capacity should be reduced about 1/3 in the Celina and Paulding soil and by 1/2 in the Ottokee sand for direct comparison with the saturation extract.

It is evident from Table 20 that accumulation of soluble salts could be a

Table 20. SPECIFIC CONDUCTANCE OF SOIL SOLUTIONS DISPLACED FROM SLUDGE AMENDED OTTOKEE SAND.[a] VALUES ARE MEANS OF DUPLICATE COLUMNS.

Exp. No.	Incubation Time (mo.)	Specific Conductance (mmho/cm)[b]					
		Field Capacity			Saturated		
		Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
I	1	1.2(0.4)	11.1(3.3)	10.4(3.1)	0.6(0.2)	6.7(2.0)	12.5(3.8)
	3	1.3(0.4)	11.8(3.5)	13.6(4.1)	0.8(0.2)	8.2(2.5)	15.0(4.5)
	6	1.7(0.5)	16.0(4.8)	18.0(5.4)	1.0(0.3)	10.3(3.1)	15.5(4.7)
II	1	0.9(0.3)	9.0(2.7)	9.5(2.9)	0.4(0.1)	1.5(0.5)	8.7(2.6)
	3	1.0(0.3)	11.3(3.4)	16.0(4.8)	0.8(0.2)	6.5(2.0)	13.3(4.0)
	6	1.6(0.5)	12.9(3.9)	20.2(6.1)	1.0(0.3)	9.5(2.9)	16.3(4.9)
III	1	1.0(0.3)	10.5(3.2)	13.5(4.1)	0.9(0.3)	6.4(1.9)	12.5(3.8)
	3	2.2(0.7)	11.9(3.6)	16.9(5.1)	0.8(0.2)	10.5(3.2)	17.0(5.1)
	6	3.4(1.0)	21.3(6.4)	16.5(5.0)	1.4(0.4)	13.2(4.0)	18.3(5.5)
IV	1	1.1(0.3)	11.1(3.3)	16.8(5.0)	0.6(0.2)	7.5(2.3)	12.5(3.8)
	3	2.0(0.6)	19.0(5.7)	21.8(6.5)	1.1(0.3)	9.5(2.9)	15.3(4.6)
	6	2.6(0.8)	17.8(5.3)	19.8(5.9)	1.4(0.4)	10.8(3.2)	17.8(5.3)
0 day		0.5(0.2)	3.3(1.0)	7.7(2.3)	0.3(0.1)	2.0(0.6)	5.2(1.6)

[a] Sludge amendments in metric tons/ha

[b] Values in ( ) are calculated osmotic pressures (atm.) using a factor of 0.3 X Specific Conductance (mmho/cm). (Jackson, 1958)

Table 21. SPECIFIC CONDUCTANCE OF SOIL SOLUTIONS DISPLACED FROM SLUDGE AMENDED GELINA SILT LOAM.[a] VALUES ARE MEANS OF DUPLICATE COLUMNS.

Exp. No.	Incubation Time (mo.)	Specific Conductance (mmho/cm)[b]					
		Field Capacity			Saturated		
		Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
I	1	1.9(0.6)	9.3(2.8)	9.5(2.9)	1.5(0.5)	6.9(2.1)	6.9(2.1)
	3	2.1(0.6)	11.3(3.4)	15.3(4.6)	1.0(0.3)	4.3(1.3)	7.5(2.3)
	6	2.8(0.8)	11.1(3.3)	14.4(4.3)	1.3(0.4)	4.3(1.3)	16.5(5.0)
II	1	2.4(0.7)	4.8(1.4)	7.2(2.2)	2.5(0.8)	4.1(1.2)	5.7(1.7)
	3	2.5(0.8)	5.0(1.5)	8.4(2.5)	2.0(0.6)	3.2(1.0)	5.9(1.8)
	6	3.2(1.0)	13.0(4.5)	10.1(3.0)	1.9(0.6)	3.0(0.9)	13.4(4.0)
III	1	2.3(0.7)	6.0(1.8)	9.1(2.7)	1.8(0.5)	3.3(1.0)	10.3(3.1)
	3	2.8(0.8)	12.6(3.8)	14.5(4.4)	1.4(0.4)	4.7(1.4)	14.3(4.3)
	6	3.8(1.1)	17.8(5.3)	21.8(6.5)	1.3(0.4)	4.3(1.3)	18.8(5.6)
IV	1	2.1(0.6)	8.0(2.4)	10.8(3.2)	2.5(0.8)	5.0(1.5)	12.5(3.8)
	3	3.2(1.0)	13.1(3.9)	16.4(4.9)	1.3(0.4)	4.4(1.3)	12.2(3.7)
	6	3.9(1.2)	16.3(4.9)	21.5(6.5)	1.2(0.4)	4.1(1.2)	14.2(4.3)
0 day		1.8(0.5)	3.3(1.0)	6.3(1.9)	1.5(0.5)	3.3(1.0)	6.0(1.8)

[a] Sludge amendments in metric tons/ha.

[b] Values in ( ) are calculated osmotic pressures (atm.) using a factor of 0.3 X Specific Conductance (mmho/cm). (Jackson, 1958)

Table 22. SPECIFIC CONDUCTANCE OF SOIL SOLUTIONS DISPLACED FROM SLUDGE AMENDED PAULDING CLAY.[a] VALUES ARE MEANS OF DUPLICATE COLUMNS.

Exp. No.	Incubation Time (mo.)	Specific Conductance (mmho/cm)[b]					
		Field Capacity			Saturated		
		Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
I	1	1.4(0.4)	1.8(0.5)	1.6(0.5)	0.8(0.2)	1.2(0.4)	2.0(0.6)
	3	0.4(0.1)	0.5(0.2)	1.8(0.5)	0.5(0.2)	1.8(0.5)	2.9(0.9)
	6	0.3(0.1)	0.6(0.2)	1.8(0.5)	1.0(0.3)	2.7(0.8)	3.2(1.0)
II	1	0.4(0.1)	0.3(0.1)	1.3(0.4)	0.6(0.2)	0.9(0.3)	1.8(0.5)
	3	0.7(0.2)	2.4(0.7)	1.9(0.6)	0.9(0.3)	2.1(0.6)	2.3(0.7)
	6	0.3(0.1)	0.5(0.2)	1.8(0.5)	0.3(0.1)	1.5(0.5)	1.8(0.5)
III	1	1.1(0.3)	1.0(0.3)	1.6(0.5)	1.0(0.3)	0.9(0.3)	1.6(0.5)
	3	1.1(0.5)	1.1(0.3)	0.8(0.2)	1.2(0.4)	0.9(0.3)	1.5(0.5)
	6	--	--	--	--	--	--
IV	1	0.9(0.3)	0.7(0.2)	0.9(0.3)	1.1(0.3)	0.7(0.2)	1.7(0.5)
	3	--	--	--	--	--	--
	6	--	--	--	--	--	--
0 day		0.5(0.2)	0.7(0.2)	2.1(0.6)	0.9(0.3)	1.0(0.3)	2.5(0.8)

[a] Sludge amendments in metric tons/ha

[b] Values in ( ) are calculated osmotic pressures (atm.) using a factor of 0.3 X Specific Conductance (mmho/cm). (Jackson, 1958)

potentially severe problem in the Ottokee sand at both sludge loading rates and at both soil moisture contents. A severe problem could also exist in the Celina soil at both loading rates at field capacity, but only at the high 224 metric ton loading rate under saturated conditions (Table 21). In contrast the accumulation of salts in the soil solution of the Paulding clay would have minimal effects on plant growth (Table 22). It should also be noted that specific conductivity of soil solutions increased with time of incubation and with increases in incubation temperature. Both observations point to the significance of the microbial population in mineralization reactions which contribute to soluble salts. Certainly high concentrations of soluble mineral nitrogen have made an appreciable contribution to the specific conductivity in the Ottokee sand and Celina silt loam.

The question might be asked, "How well can these laboratory incubation data be extrapolated to field conditions where rainfall and plant uptake would be expected to reduce accumulation of soluble salts?" As discussed previously, these data would provide information on the potential quantity of soluble salts which could be released to the surrounding soil environment during a set period of incubation. Under conditions of minimal precipitation, or in soils with an impermeable B horizon, plowsole or pan, leaching of salts from the surface soil would not occur, and the potential for salt accumulation would exist. Hinesly et al. (1972) have speculated that salt accumulation may have adversely affected corn yields on sludge treated plots during the dry conditions experienced in 1971.

Organic Matter

Soluble organic matter in soils amended with high rates of sewage sludge could be of significance as a potential pollutant of ground or surface water supplies. In addition many soluble organic compounds are capable of complexing and solubilizing heavy metal ions which would alter the plant availability and downward movement of these ions in the soil.

The organic matter content of the displaced soil solutions from all three soils averaged for all four temperature cycles are shown in Table 24. High concentrations of soluble organic matter were present in the soil solution from the sludge amended Ottokee sand incubated at field capacity. The amount of organic matter was directly related to the rate of sludge amendment.

Moderate levels of soluble organic matter were present in the Ottokee sand under saturated conditions, and in the Celina silt loam soil at both moisture contents. The concentration of soluble organic matter in the Paulding clay was 10 to 30 times lower than that in the Ottokee sand and increased only slightly above the unamended control soils. Also note that the soluble organic matter in sludge amended soils arises primarily from the sludge itself (see 0-day concentrations of soluble organic matter) and decreases slightly with incubation. The one exception to this is in the Ottokee sand, incubated at field capacity. Here an increase in soluble organic matter occurred with incubation.

These data suggest that soluble organic matter could present a pollution hazard to ground water in sludge amended coarse textured soils like the Ottokee. Accumulation of soluble organic matter should not be a pollution

Table 24. ORGANIC MATTER CONTENT OF SOIL SOLUTIONS DISPLACED FROM SLUDGE AMENDED SOILS.

		Organic Matter Content (mg/ml)[a]					
		Field Capacity			Saturated		
Soil	Incubation Time	Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
Ottokee Sand	0 day	0.27	4.6	27.8	0.24	4.3	8.3
	1 mo.	0.29	26.5	93.6	0.26	3.6	8.4
	3 mo.	0.34	48.9	95.0	0.34	3.6	7.3
	6 mo.	0.40	33.5	104.3	0.27	2.2	7.9
Celina Silt Loam	0 day	0.35	6.3	9.8	0.25	4.4	7.8
	1 mo.	0.53	3.7	7.8	0.54	3.2	5.9
	3 mo.	0.43	4.5	10.4	0.54	4.3	6.6
	6 mo.	0.49	3.5	8.8	0.54	3.5	5.5
Paulding Clay	0 day	0.17	0.35	0.58	0.16	0.20	0.47
	1 mo.	0.41	0.36	0.54	0.33	0.28	0.45
	3 mo.	0.29	0.21	0.66	0.25	0.27	0.34
	6 mo.	0.13	0.16	0.50	0.15	0.17	0.35

[a] Values given are the means for displaced soil solutions from duplicate columns for Experiments I-IV.

hazzard in finer textured soils such as the Celina or Paulding where adsorption of organic matter on soil colloids would prevent appreciable downward leaching. The concentration of soluble organic matter found in the soil solution of the Celina silt loam could, however, significantly influence the solubility and mobility of heavy metal ions.

Nitrogen Transformations

Nitrogen reactions in sludge amended soil are of great significance to the success of land disposal of sewage sludges. Accumulation of nitrate in the soil at concentrations greater than that which can be utilized by the associated crop or vegetation, could result in nitrate movement into ground water. Hinsely et.al. (1972) have proposed that excessive formation of nitrate from sewage sludge nitrogen is the factor most likely to determine the maximum rate of sludge additions to soils.

In this report, data are presented on the distribution of organic, NH_4^+, NO_2^-, and NO_3^- nitrogen in the displaced soil solution from the Ottokee sand and Celina silt loam; and for NH_4^+ and NO_3^- nitrogen from the Paulding clay. In the latter soil preliminary analysis of the soil solutions showed both NO_2^- and organic nitrogen to be absent or present in negligible quantities. Nitrogen data reported in Figures 26 to 43 show the differences in soil nitrogen associated with the differing sludge loading rates, soil moisture contents, and temperatures of incubation (Expt. I to IV). Although these data are reported in ug N/ml of the soil solution, a conversion to ug/g dry soil can be made by multiplying the following conversion factors.

Ottokee sand	-field capacity	0.080
	-saturated	0.200
Celina silt loam	-field capacity	0.215
	-saturated	0.350
Paulding clay	-field capacity	0.329
	-saturated	0.620

Each soil exhibited characteristic soluble nitrogen accumulation curves. For example, ammonium nitrogen and soluble organic nitrogen were the most significant forms of nitrogen found in sludge amended Ottokee sand after 1 month's incubation. It seems obvious from these data that some component of sewage sludge inhibited nitrification while allowing ammonification to proceed. The duration of this inhibition of nitrification varied and was more prolonged at the higher sludge loading rates, and at the cooler incubation temperatures. As would be expected the concentration of ammonium nitrogen during subsequent incubation was inversely related to nitrate accumulation. This was also true to a certain degree with soluble organic nitrogen. There was no absolute evidence of denitrification in the Ottokee sand although the leveling off of the rate of accumulation of nitrate during the last three month's incubation could suggest at least some loss of nitrate through denitrification.

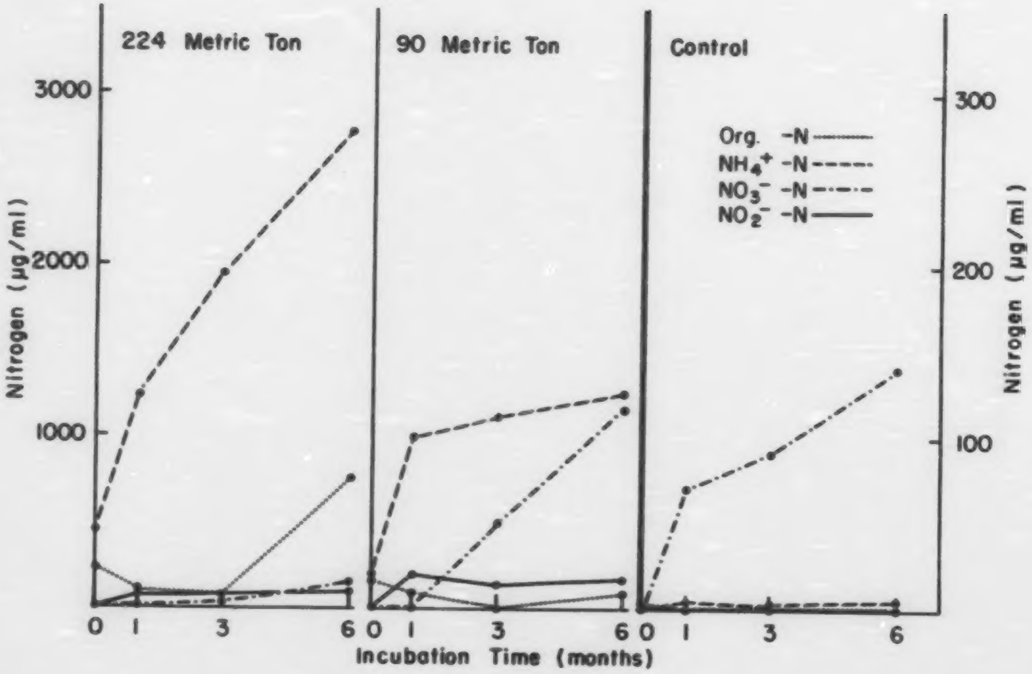

Figure 26. Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated at field capacity (Experiment I, autumn-winter).

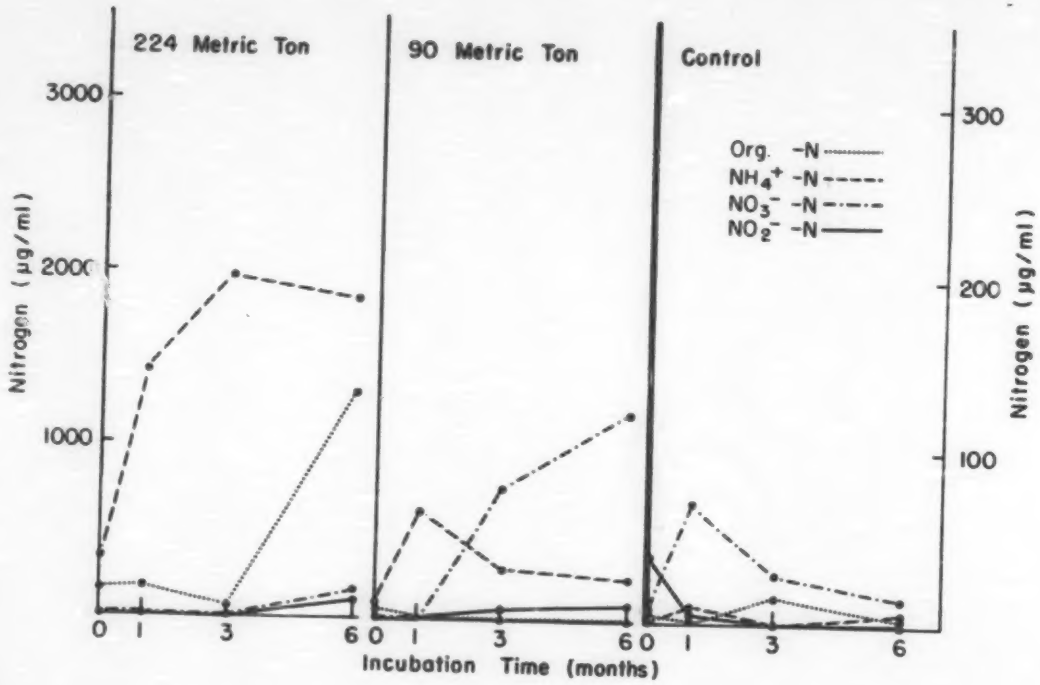

Figure 27. Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated under saturated conditions (Experiment I, autumn-winter).

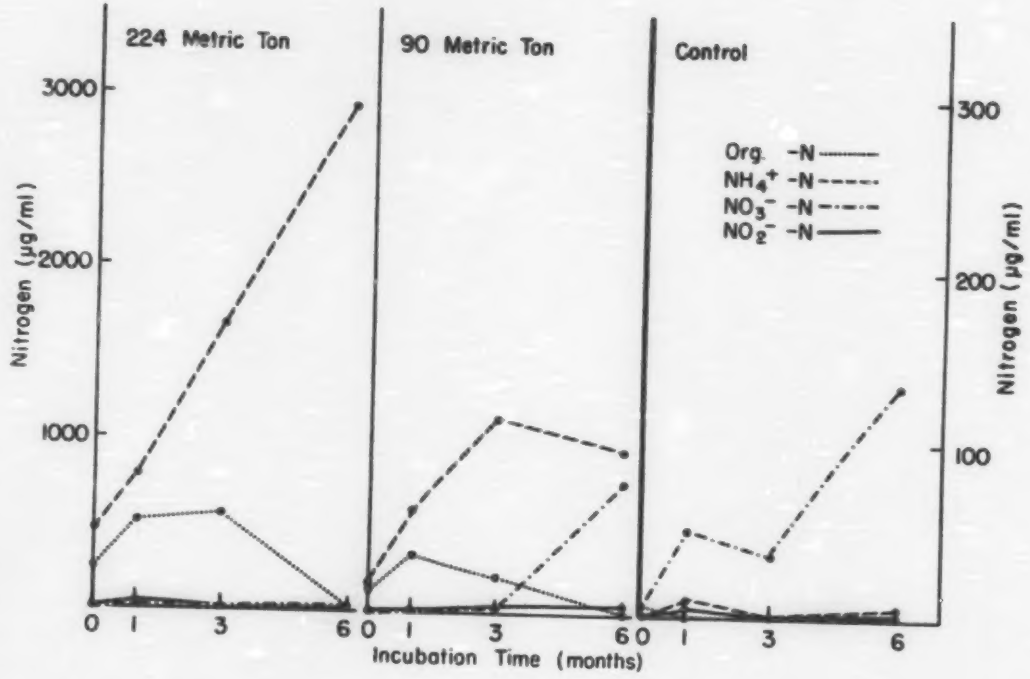

Figure 28. Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated at field capacity (Experiment II, winter-spring).

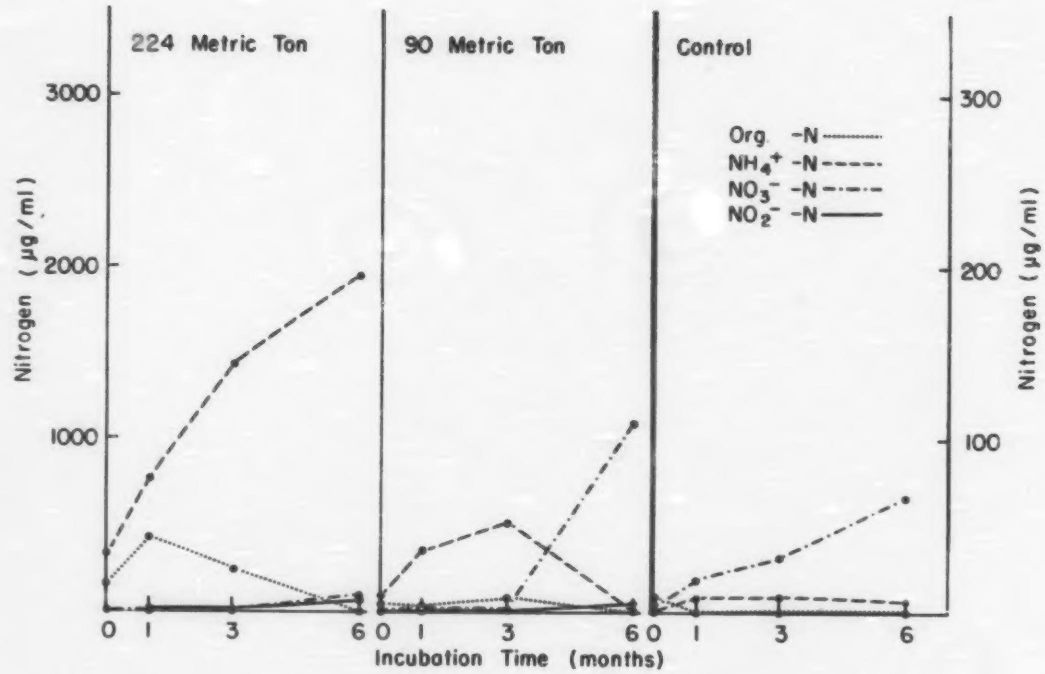

Figure 29. Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated under saturated conditions (Experiment II, winter-spring).

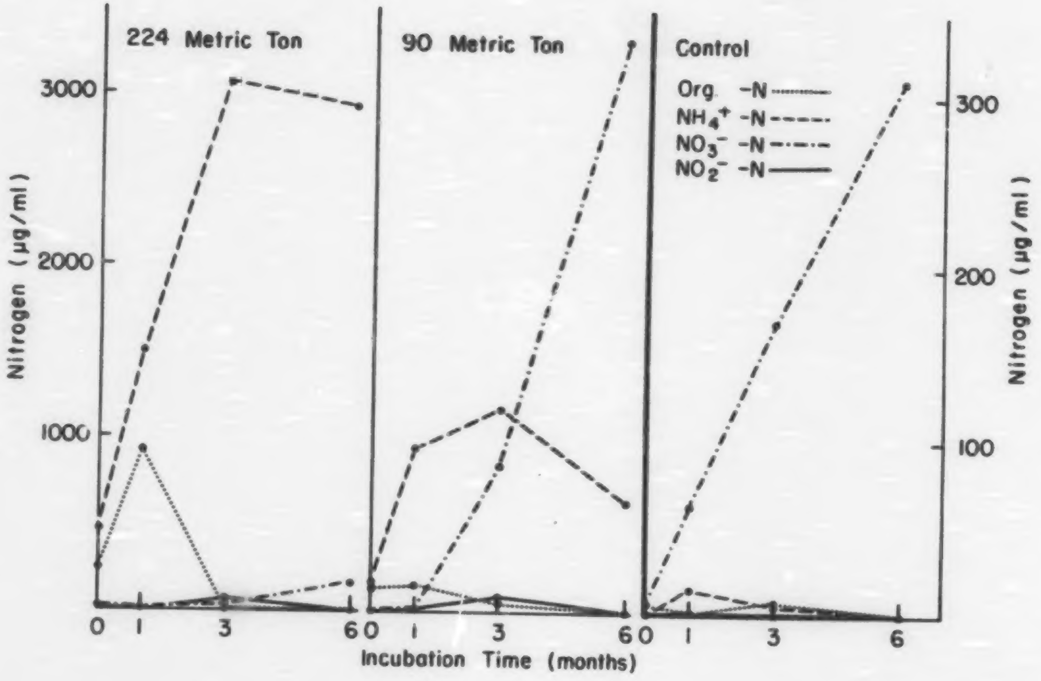

Figure 30. Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated at field capacity (Experiment III, spring-summer).

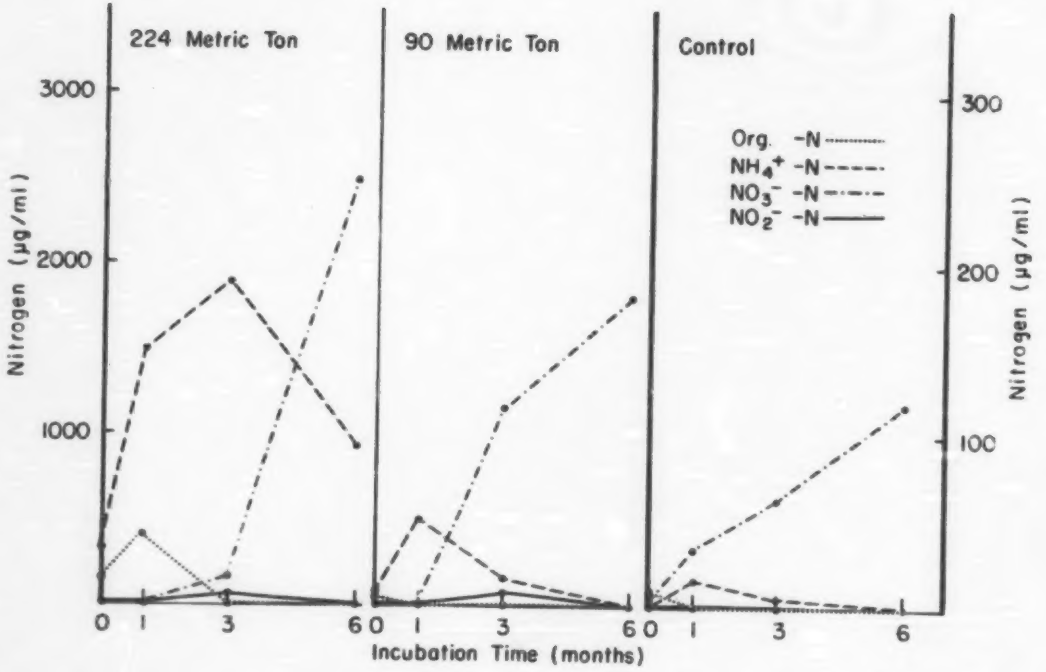

Figure 31. Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated under saturated conditions (Experiment III, spring-summer).

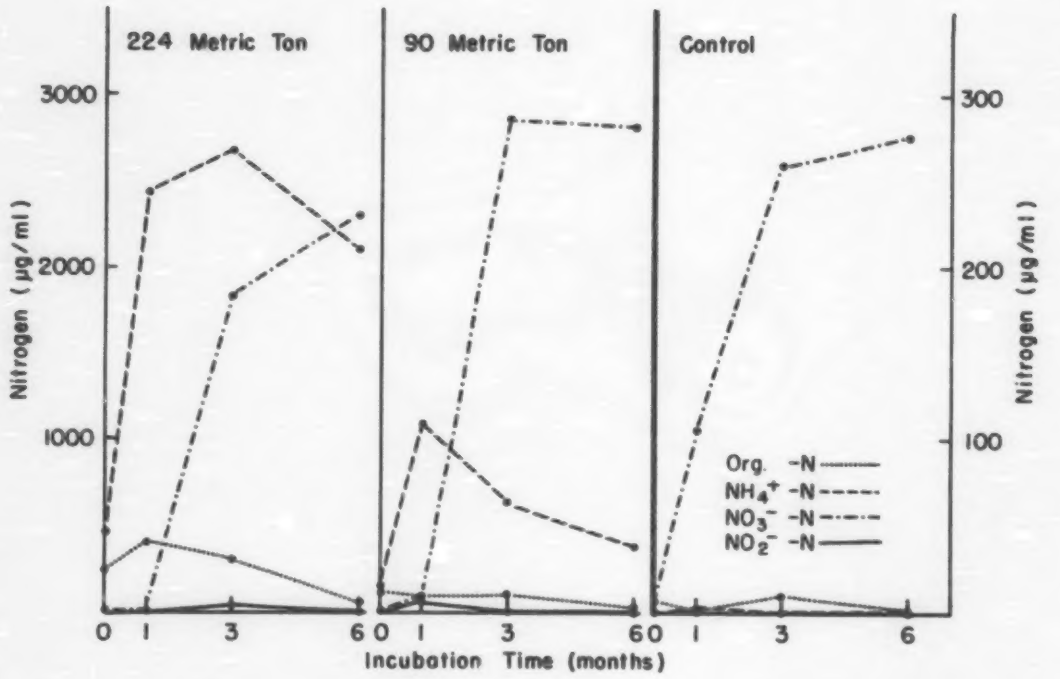

Figure 32. Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated at field capacity (Experiment IV, summer-autumn).

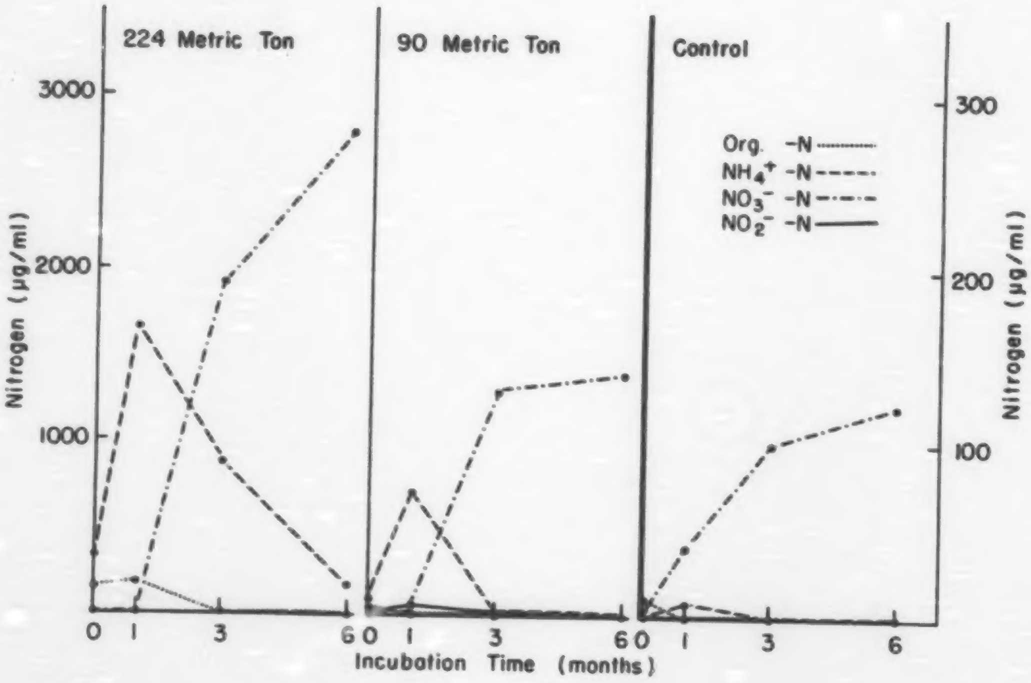

Figure 33. Soluble nitrogen in displaced soil solutions from sludge amended and control Ottokee sand incubated under saturated conditions (Experiment IV, summer-autumn).

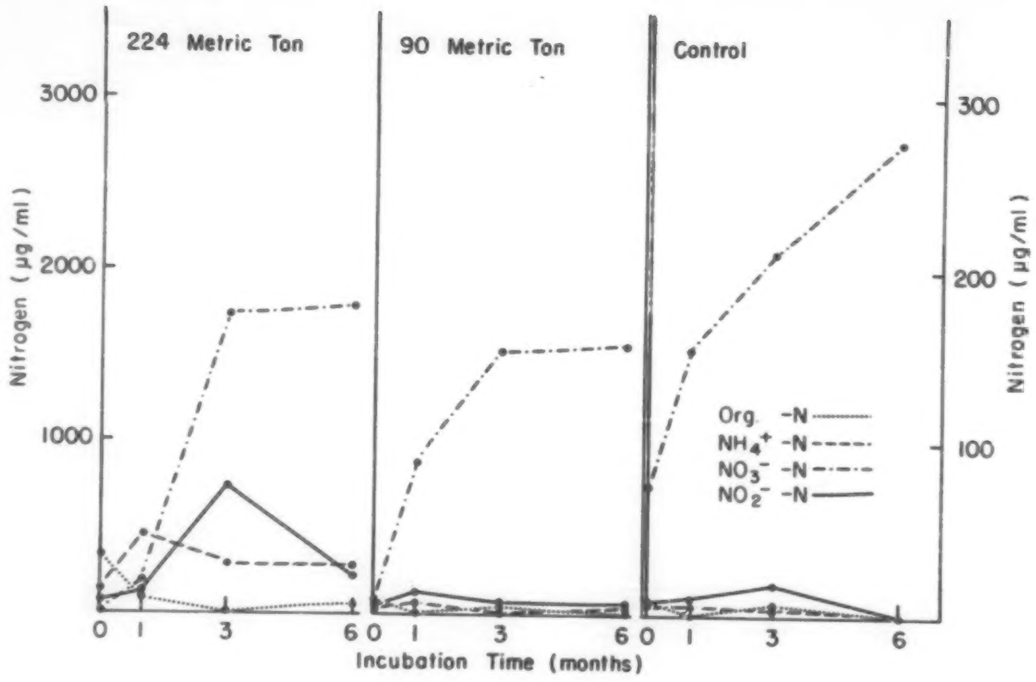

Figure 34. Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated at field capacity (Experiment I, autumn-winter.

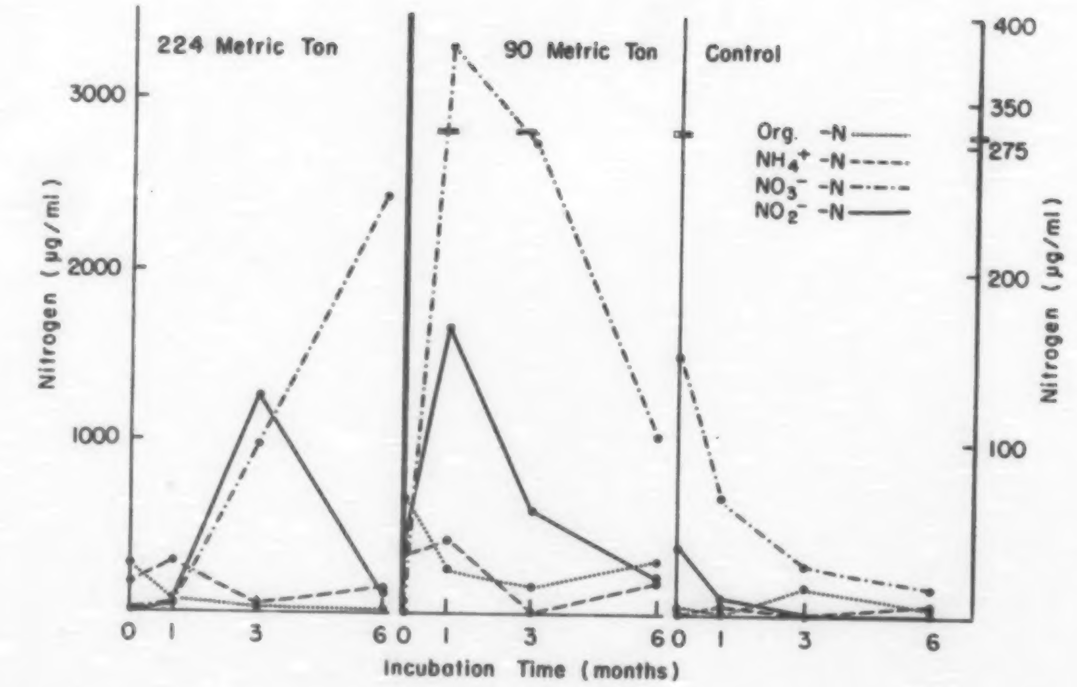

Figure 35. Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated under saturated conditions (Experiment I, autumn-winter).

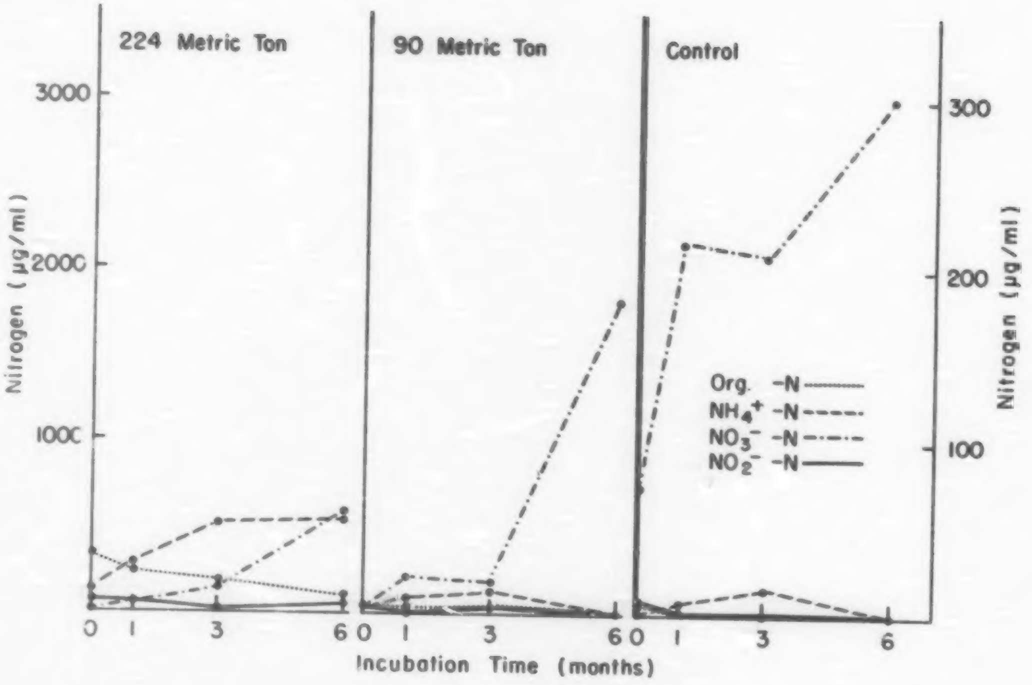

Figure 36. Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated at field capaicty (Experiment II, winter-spring).

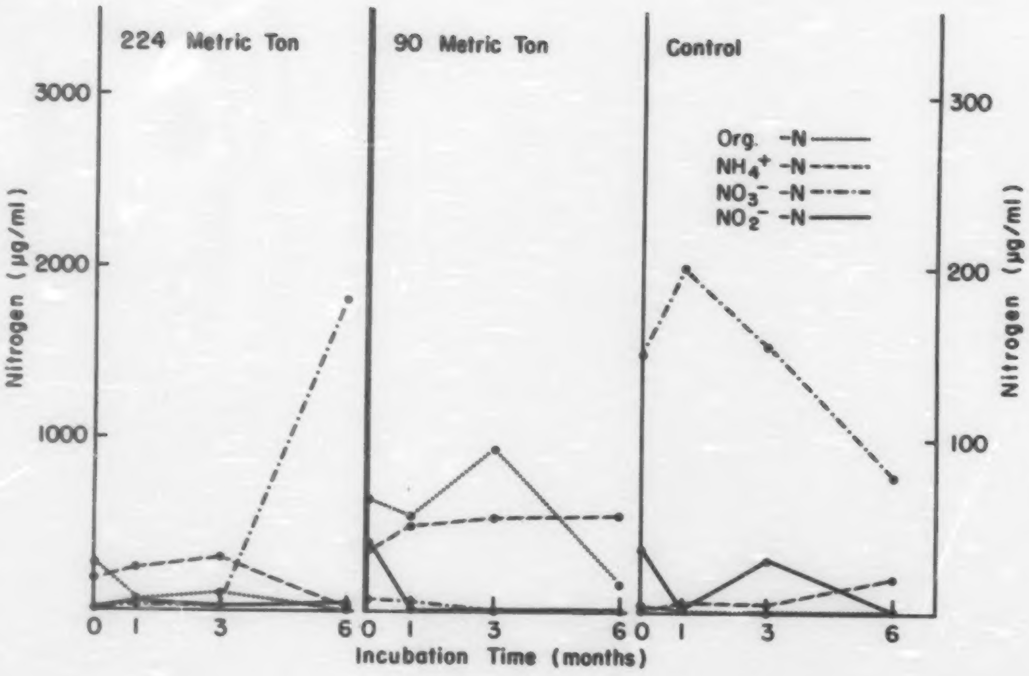

Figure 37. Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated under saturated conditions (Experiment II, winter-spring).

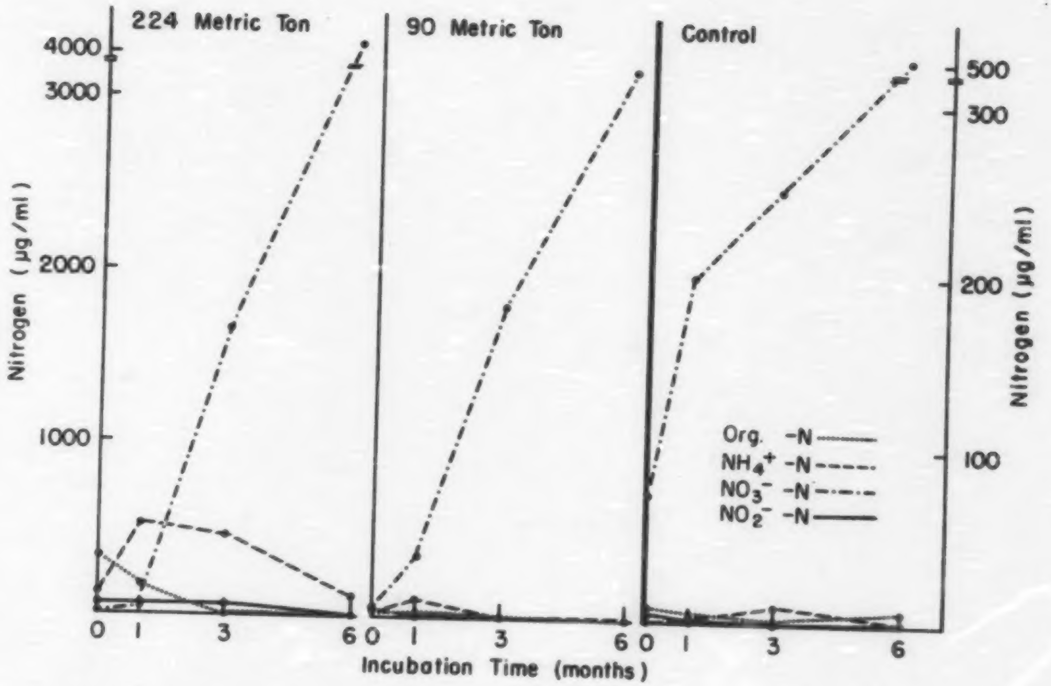

Figure 38. Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated at field capacity (Experiment III, spring-summer).

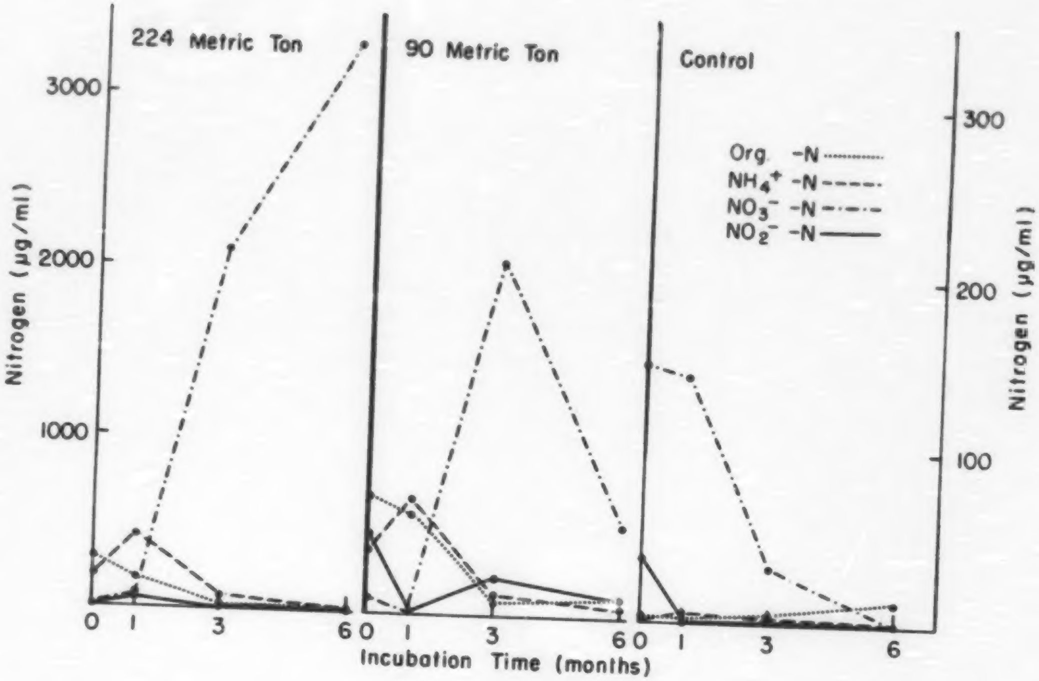

Figure 39. Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated under saturated conditions (Experiment III, spring-summer).

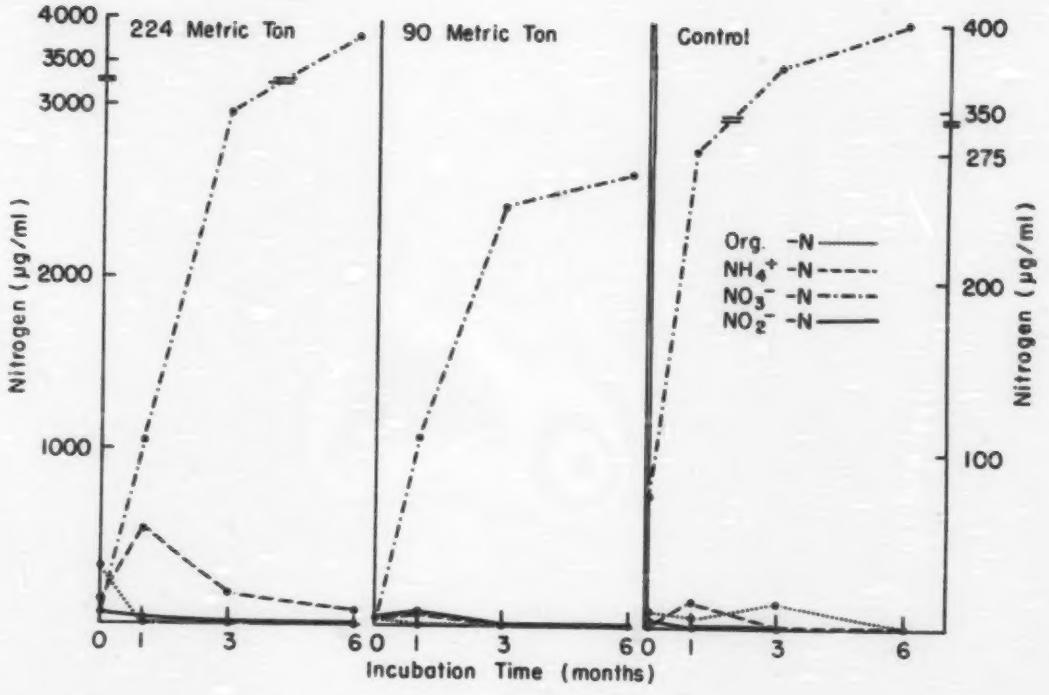

Figure 40. Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated at field capacity (Experiment IV, summer-autumn).

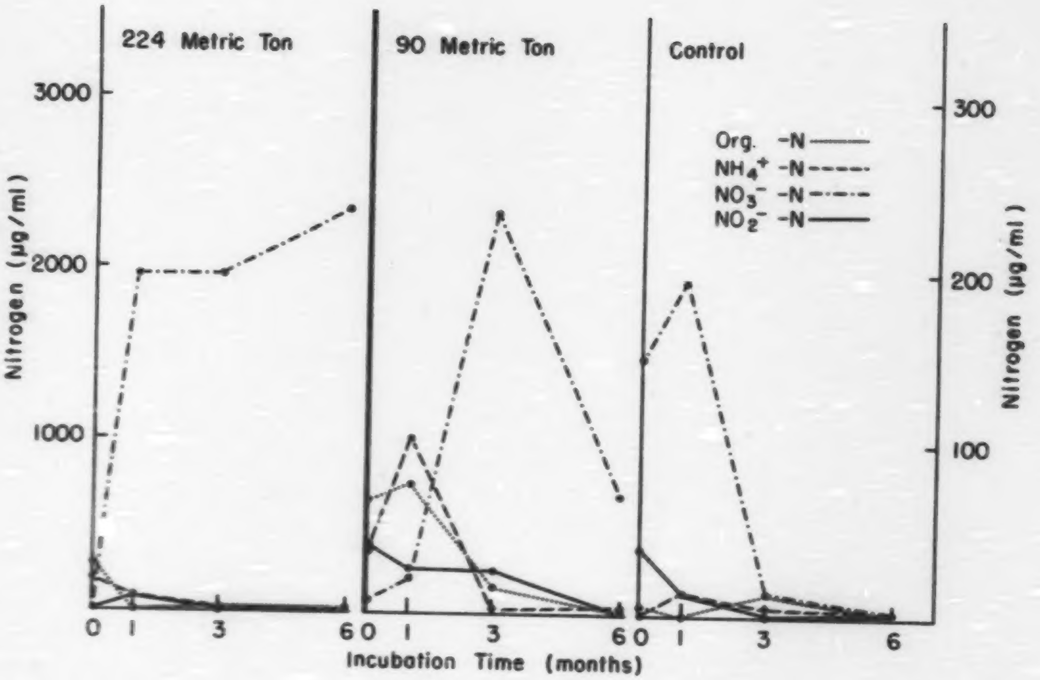

Figure 41. Soluble nitrogen in displaced soil solutions from sludge amended and control Celina silt loam incubated under saturated conditions (Experiment IV, summer-autumn).

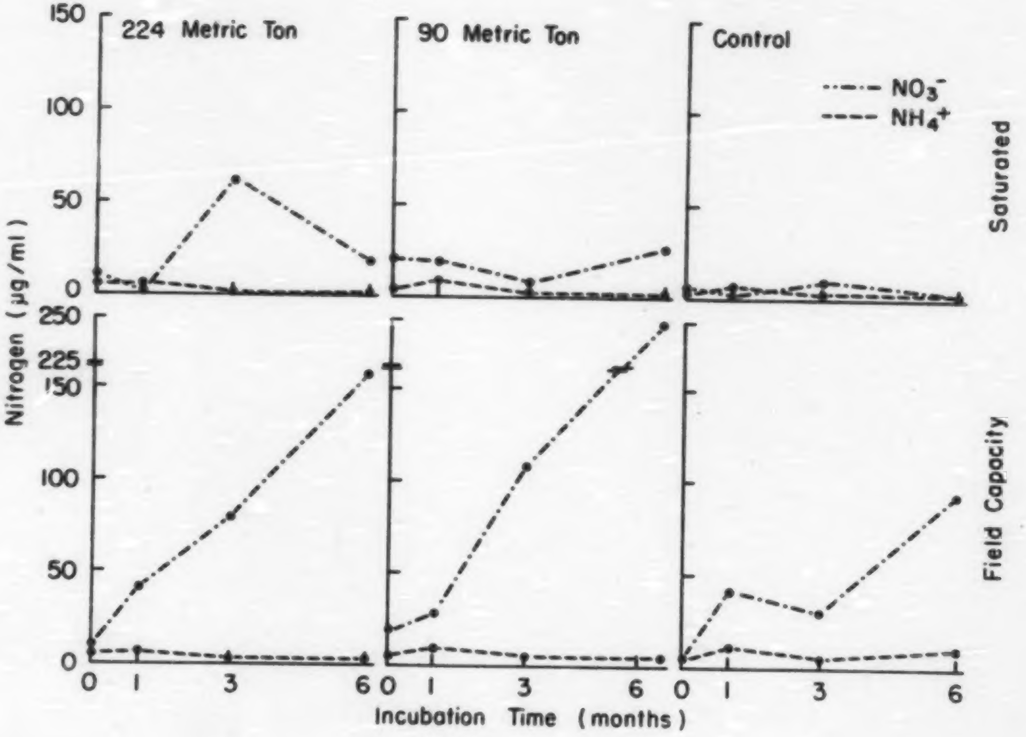

Figure 42. Soluble ammonium and nitrate nitrogen in displaced soil solutions from sludge amended and control Paulding clay (Experiment I, autumn-winter).

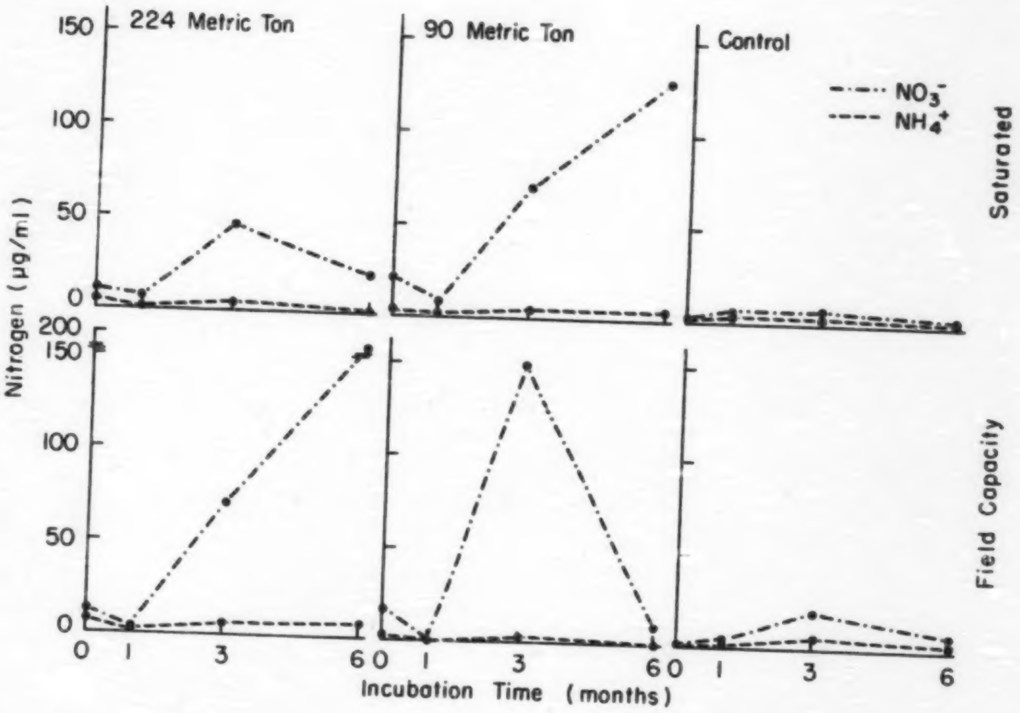

Figure 43. Soluble ammonium and nitrate nitrogen in displaced soil solutions from sludge amended and control Paulding clay (Experiment II, winter-spring).

The pattern of nitrogen accumulation in the Celina silt loam differed from the Ottokee sand in several ways. First, the concentration of ammonium nitrogen never approached that of the Ottokee sand, although values as high as 500 ug/ml were found early in the incubation period. Lower levels of NH_4^+ in the soil solution would be expected because of the higher cation exchange capacity of the Celina soil. Second, soluble organic nitrogen normally reached insignificant levels after 1 months incubation. Third, nitrite was found in a few of the soil columns above the levels which might be considered normal. Fourth, nitrification was more extensive in both the sludge amended and control soils at field capacity. However, there was still evidence of a partial inhibition of nitrification of about a 1 month's duration. Lastly, the lower concentration of nitrate in the saturated soils, as well as the decreases in nitrate with time of incubation provide presumptive evidence for active biological denitrification.

Quantities of ammonium and nitrate nitrogen in the displaced soil solution from the Paulding clay are plotted in Figures 42 and 43. Data is not given for Experiments III and IV because these studies were terminated before the entire 6 month incubation period was complete. Nitrate nitrogen was found to be the only significant form of soluble nitrogen in the Paulding clay. Ammonium nitrogen was present at a concentration of less than 10 ug/ml. Low concentration of soluble NH_4^+ would be expected in soils with a high exchange capacity such as the Paulding. It is also apparent that the concentration of soluble nitrogen found is considerably lower than in comparable treatments for the Celina and Ottokee soils. In general and as expected, the quantity of nitrate found when the soil was incubated at field capacity was greater than under saturated conditions. This could reflect either decreased nitrification or increased denitrification under conditions of reduced aeration. As shown previously for the other two soils, nitrification was again inhibited for about a 1 month period.

An attempt was made to estimate the percentage of the sludge organic nitrogen which was found in soluble form after 6 months incubation. The calculated values shown in Table 25 are corrected for soluble nitrogen in the control soil and for ammonium nitrogen originally present in the sludge. Data were not given for the Celina and Paulding soils incubated under saturated conditions since the quantity of soluble nitrogen under these conditions of reduced aeration would not be representative. Mineralized nitrogen appearing in the soil solution ranged from 0.0 to 32.0% of the organic nitrogen present in the sludge. The apparent lack of mineralization in the Paulding clay must be interpreted by considering that nitrate accumulation will be the net result of the opposing processes of nitrification and denitrification. Net accumulation could be expected to be low in a fine textured poorly aerated soil such as the Paulding clay. In addition, these data do not allow us to determine the actual mineralization of sludge organic nitrogen, since appreciable quantities of NH_4^+ nitrogen may be on the soil exchange sites. The lower value in the Ottokee sand is a result of reduced microbial activity because of limiting moisture. The higher value in the Celina silt loam probably represents a maximum value associated with optimal conditions for nitrification with little if any denitrification. Whether or not this value represents the actual mineralization of nitrogen would depend on

Table 25. PERCENTAGE OF THE ORGANIC NITROGEN OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE APPEARING IN THE SOIL SOLUTION AFTER 6 MONTHS INCUBATION.

Soil & Treatment	Sludge amendments (Metric Ton/ha)	
	224	90
Ottokee Sand- field capacity (Data from Figure 32)	3.3%	11.8%
Ottokee Sand-Sat. (Data from Figure33)	10.0%	13.4%
Celina silt loam-field capacity (Data from Figure 40)	16.4%	32.0%
Paulding clay- field capacity (Data from Figure 42)	0.0%	0.0%

the amount of NH_4^+ remaining on the exchange sites. Since no attempt was made to measure exchangeable ammonium we cannot answer this question.

Spectrographic Analyses

The last group of analyses carried out on the displaced soil solution were those for various nutrient cations and anions. Although spectrographic analysis was routinely made for 15 elements (P, K, Ca, Mg, Na, Si, Mn, Fe, B, Cu, Zn, Al, Sr, Ba, and Mo) only 6 elements (Ca, Mg, Na, Cu, Mn, and Zn) showed differences in concentration which were attributable to the various soil treatments, Three of these, Cu, Mn, and Zn are normally present in soil solutions below the limit of detection by spectrographic analysis. The addition of sewage sludge to the soil and concomitant incubation and sludge degradation increased the concentration of these elements sufficiently to exceed the limits of detection. Solution phosphorus was not increased, even though rather large quantities of phosphorus were added with the sludge amendments. This fact illustrates the effectiveness of adsorption and precpitation reactions in reducing the concentration of soluble phosphorus in soils.

Data showing the concentration of Ca, Mg, and Na in the displaced soil solution are given in Tables 26, 27, and 28. It is apparent that the addition of sewage sludge increases the concentration of all three elements appreciably. The concentration of all three elements, but particularly Ca also increased with incubation time, which may reflect altered solubility and mineralization associated directly or indirectly with microbial activity eg. increase in H^+ associated with nitrification. The soil solution from the Paulding clay was considerably more dilute than the solutions from the Celina or Ottokee soils which were about equal. We can expect that the large amounts of Na, Mg and Ca contained in sewage sludge will participate in normal exchange reactions in soil. Once steady state equilibrium is reached rather large concentrations of these elements may be carried downward in the soil profile into groundwater supplies.

The concentrations of Zn, Cu and Mn in the displaced soil solutions are shown in Tables 29, 30, and 31. As mentioned previously in this section, these elements are seldom present in the soil solutions at concentrations detectable by an emission spectrograph without prior concentration. The presence of detectable concentrations of Zn, Cu and Mn in the soil solution from sludge amended soils reflects the influence of sewage sludge in supplying these elements to the soil. The concentration in solution at any one time will reflect various microbial reactions such as oxidation reduction, mineralization, production of soluble chelates, or indirectly by changing the soil pH.

Manganese solubility is increased by conditions which would enhance the reduction of MN^{4+} to the more soluble Mn^{2+} . In this study, reducing conditions associated with saturated moisture conditions and increased levels of organic substrate would seem to explain the increased presence of Mn in the soil solutions (Table 31). Soluble Cu (Table 30) was highest in the Ottokee sand which also had the highest concentration of soluble organic matter (Table 24). Increased solubility of Zn may reflect both the accumulation of soluble organic matter as well as a reduction of soil pH associated with active nitrification particularly in the Celina silt

Table 26. CONCENTRATION OF Ca IN DISPLACED SOIL SOLUTIONS FROM SLUDGE AMENDED SOILS.

Soil	Incubation Time (Mo.)	Concentration of Ca (mg/ml)[a]					
		Field Capacity			Saturated		
		Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
Ottokee Sand	0	200	2413	2370	200	725	1465
	1	318	1745	1010	213	1940	1260
	3	476	3061	1311	254	5561	2773
	6	1111	5156	1965	445	7146	4485
Celina Silt Loam	0	940	1700	3645	1210	1975	2140
	1	1034	6346	3715	1198	3862	4632
	3	1280	8083	6641	755	5505	5665
	6	2052	7240	6695	589	3130	8620
Paulding Clay	0	200	200	1015	280	355	1965
	1	651	583	826	621	508	1169
	3	728	1370	1298	557	930	1918
	6	218	250	1648	330	1330	2693

[a] Values given are the means for displaced soil solutions from duplicate columns for Experiments I-IV.

Table 27. CONCENTRATION OF Mg IN DISPLACED SOIL SOLUTIONS FROM SLUDGE AMENDED SOILS.

Soil	Incubation Time (Mo)	Concentration of Mg (mg/1)[a]					
		Field Capacity			Saturated		
		Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
Ottokee Sand	0	125	1343	1340	70	405	985
	1	256	1383	780	120	1274	1686
	3	388	7601	978	294	2393	2029
	6	825	2465	970	310	2935	2472
Celina silt loam	0	448	1335	1820	700	1560	2290
	1	415	2236	2549	580	1676	2121
	3	561	2314	2529	365	2070	2276
	6	746	3096	2586	248	2200	3008
Paulding Clay	0	60	100	680	220	240	1015
	1	390	399	533	389	301	719
	3	328	660	823	283	568	1067
	6	83	100	883	260	718	1260

[a] Values given are the means for displaced soil solutions from duplicate columns for experiments I-IV.

Table 28. CONCENTRATION OF Na IN DISPLACED SOIL SOLUTIONS FROM SLUDGE AMENDED SOILS.

Soil	Incubation Time (mo)	Concentration of Na (mg/l)[a]					
		Field Capacity			Saturated		
		Control	90 Ton	224 Ton	Control	90 Ton	224 Ton
Ottokee Sand	0	70	170	195	55	135	165
	1	< 20	201	208	< 20	164	210
	3	< 20	220	223	< 20	175	241
	6	< 20	235	241	< 20	185	246
Celina Silt Loam	0	30	125	170	< 20	110	185
	1	24	150	201	34	125	219
	3	23	134	209	35	145	185
	6	35	178	244	24	140	245
Paulding Clay	0	53	50	80	65	55	85
	1	49	44	49	38	50	65
	3	52	82	100	77	82	103
	6	48	50	90	48	105	100

[a] Values given are the means for displaced soil solutions from duplicate columns for experiments I-IV.

Table 29. CONCENTRATION OF Zn IN DISPLACED SOIL SOLUTIONS FROM SLUDGE AMENDED SOILS.

Soil	Incubation Time (Mo.)	Concentration of Zn (mg/1)[a]			
		Field Capacity		Saturated	
		90 Ton	224 Ton	90 Ton	224 Ton
Ottokee Sand	0	ND	ND	ND	ND
	1	2	3	2	5
	3	3	4	10	5
	6	7	1	13	10
Celina Silt Loam	0	ND	ND	ND	ND
	1	3	3	1	9
	3	9	11	3	11
	6	9	11	1	19
Paulding Clay	0	ND	ND	ND	ND
	1	ND	1	1	3
	3	2	1	1	1
	6	ND	5	5	5

ND= Concentration below detection limit of the emission spectrograph

[a] Values given are the means for displaced soil solutions from duplicate columuns for experiments I-IV.

Table 30. CONCENTRATION OF Cu IN DISPLACED SOIL SOLUTIONS FROM SLUDGE AMENDED SOILS.

Soil	Incubation Time (Mo.)	Concentration of Cu (mg/1) [a]			
		Field Capacity		Saturated	
		90 Ton	224 Ton	90 Ton	224 Ton
Ottokee Sand	0	1	2	ND	ND
	1	2	7	1	2
	3	2	10	1	4
	6	3	12	1	4
Celina Silt Loam	0	ND	ND	ND	ND
	1	1	2	1	1
	3	1	2	1	ND
	6	1	2	ND	ND
Paulding Clay	0	ND	ND	ND	ND
	1	ND	ND	ND	1
	3	1	6	ND	ND
	6	ND	ND	1	2

ND = Concentration below detection limits of the emission spectrograph

[a] Values given are the means for displaced soil solutions from duplicate columns for experiments I-IV.

Table 31. CONCENTRATION OF Mn IN DISPLACED SOIL SOLUTIONS FROM SLUDGE AMENDED SOILS.

Soil	Incubation Time (Mo)	Concentration of Mn (mg/l)[a]			
		Field Capacity		Saturated	
		90 Ton	224 Ton	90 Ton	224 Ton
Ottokee Sand	0	5	3	3	4
	1	6	3	7	6
	3	11	4	47	12
	6	21	3	68	31
Celina Silt Loam	0	6	9	5	5
	1	5	20	53	40
	3	4	28	42	56
	6	7	49	25	76
Paulding Clay	0	3	5	3	17
	1	4	5	4	8
	3	7	7	7	12
	6	3	7	7	17

[a] Values given are the means for displaced soil solutions from duplicate columns for experiments I-IV.

loam. The significance of increased solubility of Zn, Mn, and Cu on the uptake of these elements by Kentucky 31 Fescue will be discussed in the next section of this report.

PHASE 4; EFFECTS OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ON KENTUCKY 31 FESCUE

One highly significant factor affecting the utilization of soil for disposal of sewage sludges is the effect of the sludge on the growth of higher plants. Potential problems might arise because of phytotoxic effects associated with sludge organic matter itself, organic degradation products, changes in the soil microflora, soluble salts, excess ammonium or nitrite nitrogen, ion imbalances in soil, or toxic concentrations of heavy metals.

Phytotoxic effects might be expressed by one or more plant responses such as reduced germination, sub-optimal plant growth, or changed chemical composition. The studies reported in this section evaluated the influence of anaerobically digested sewage sludge on the germination, growth, and chemical composition of Kentucky 31 Fescue. Evaluations were made on sewage sludge amended soils after 1, 3 and 6 month incubation periods at differing soil moisture contents and temperatures. Data summarizing the effects of anaerobically digested sewage sludge on germination of Kentucky 31 Fescue are shown in Table 32. Values for percentage germination for individual monthly experiments were often highly variable with no consistent trends associated with either the length of incubation or temperatures during incubation. Mean values for germination for each 6 month experiment, as well as the grand means, did show some differences, however. In general, germination of fescue in sludge amended soils incubated under saturated moisture conditions was as good or better than unamended control soils, The only pronounced negative effect noted in saturated soils was in the Ottokee sand at the 224 metric ton loading rate. Reduced germination under these conditions was probably related to the high osmotic pressure of the soil solution (See Table 20) or to the influence of sludge colloids in altering the imbibition of H_2O by the germinating seeds. The improvement in germination for the 90 metric ton sludge amendments in the Celina and Paulding soils under saturated conditions was probably related to the amelioration of adverse conditions associated with excess H_2O by soluble salts or sludge colloids.

The same two factors noted above, alone or in combination, are probably responsible for the reduced germination of Kentucky 31 Fescue in sludge amended soils kept at field capacity. Severe reductions in germination in the Paulding soil cannot be associated with the osmotic pressure of the soil solution (See Table 22) and may be related to the energy by which H_2O is held by sludge colloids.

Excellent germination of Kentucky 31 Fescue in sludge amended soils under saturated conditions certainly suggests that anaerobically digested sewage sludge does not directly affect germination. Sludge induced secondary effects on water availability to germinating seeds because of soluble salts or sludge colloids may be significant, however.

Table 32. EFFECT OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ON GERMINATION OF KENTUCKY 31 FESCUE IN SLUDGE AMENDED SOILS. DATA EXPRESSED AS % OF CONTROL

Exp. No.	Incub. Time (mo.)	Ottokee Sand				Celina silt loam				Paulding Clay			
		Sludge (metric ton/ha)				Sludge (metric ton/ha)				Sludge (metric ton/ha			
		90T-FC	90T-Sat.	224T-FC	224T-Sat.	90T-FC	90T-Sat.	224T-FC	224T-Sat.	90T-FC	90T-Sat.	224T-FC	224T-Sat.
I	1	86	91	46	30	106	89	87	105	82	107	71	103
	3	108	100	72	94	101	110	97	99	33	96	37	50
	6	68	98	76	92	103	112	104	97	65	124	46	104
Mean		87	96	65	52	103	104	99	100	60	109	51	86
II	1	82	92	76	81	95	94	69	74	79	93	62	113
	3	68	102	50	82	91	98	71	95	76	110	45	88
	6	64	102	63	93	110	94	88	104	74	102	52	89
Mean		71	99	63	85	99	95	76	91	76	102	53	97
III	1	59	94	58	87	101	83	89	86	86	145	66	146
	3	54	85	94	94	97	97	70	110	81	96	36	63
	6	116	100	107	88	85	122	81	89	-	-	-	-
Mean		76	93	86	90	94	101	80	95	84	121	51	105
IV	1	96	103	92	93	93	97	100	87	94	135	67	130
	3	114	113	72	95	94	98	69	94	-	-	-	-
	6	16	92	32	84	17	146	14	132	-	-	-	-
Mean		75	103	65	91	68	112	61	104				
Grand Mean		77	98	70	80	91	103	79	98	74	112	53	98
0 day		89	95	64	65	88	84	90	65	62	106	27	98

Data on the effect of anaerobically digested sludge on the dry matter yield of 6 week old Kentucky 31 Fescue are shown in Tables 33 and 34. It is of particular significance that sludge amended soils usually gave higher dry matter yields of fescue than control soils (Ratios>10 in Table 33). The exceptions can in most cases be related directly to poor seed germination for reasons discussed previously (See germination data, Table 32). Likewise, there were many instances where positive yield responces occurred in sludge treatments even with reduced germination.

Fescue growing in sludge amended saturated soils produced considerably more dry matter than those maintained in soils at field capacity. Visual observations of fescue growing in sludge amended soils at field capacity confirmed that conditions were less than desirable for maximum growth. Typical plants were bluish-green, stunted, with necrotic leaf tips. In contrast fescue growing in saturated soils was dark green, vigorous, with no apparent necrosis or chlorosis, It should be emphasized, however, that saturation of the soils in the growth chamber did not result in as poor root aeration as would be true under natural soil conditions. Poor plant growth in the sludge amended Ottokee and Celina soils at field capacity could very well be associated with salt accumulation to toxic levels. Specific conductance greater than 4 mmho/cm are generally considered to restrict plant yields. Measured specific conductance values ranging between 11.1 and 21.8 mmhos/cm were obtained for displaced soil solutions from these two soils (Tables 20 and 21). However, salt accumulation to toxic levels can not be the total reason since specific conductance greater than 10 mmho/cm was rather commonplace in even the saturated soils in 224 ton sludge amended soils without noticeable effects on plant growth. Furthermore, the soil solutions displaced from the Paulding clay had specific conductances below 4 mmho/cm but plant growth was still affected adversely in soil maintained at field capacity.

The fertilizer value of anaerobically digested sewage sludge is readily apparent if one looks at the yield response to sludge amendments in the soils maintained at saturated moisture conditions. However, it also appears that near maximum yields were attainable at the lower 90 metric ton/ha amendment with little yield increase attributable to the 224 metric ton/ha amendment.

Plant analysis provided information on ion uptake by Kentucky 31 Fescue from sludge amended soils after incubation for 1, 3 and 6 months. Nitrogen and sulfur data are summarized in Table 35. These data are incomplete because growth of fescue was so poor in many of the treatments that insufficient plant material was available for one or more of the analyses. Not surprisingly plant nitrogen and sulfur were directly related to the quantity of sludge added. Plant nitrogen was also lower in the saturated soils than in soils incubated at field capacity. This may in part reflect the dilution effect within the plant itself because of the better growth in saturated soils compared to those kept at field capacity. Perhaps more important, however, is the reduced availability of soluble nitrogen in saturated soils because of an increase in biological denitrification, and reduced nitrification.

Phosphorus was also higher in Kentucky 31 Fescue grown in sludge amended soils than in control soils (Figure 44). The one exception was plants grown

Table 33. EFFECT OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ON DRY MATTER YIELD OF KENTUCKY 31 FESCUE. DATA EXPRESSED AS RATIO $\frac{\text{SLUDGE TRT.}}{\text{CONTROL}}$

Exp. No.	Incub. Time (Mo.)	Ottokee Sand Sludge (metric ton/ha) 90T-FC	 90T-Sat.	 224T-FC	 224T-Sat.	Celina Silt Loam Sludge (metric ton/ha) 90T-FC	 90T-Sat.	 224T-FC	 224T-Sat.	Paulding Clay Sludge (metric ton/ha) 90T-FC	 90T-Sat.	 224T-FC	 224T-Sat.
I	1	4.3	16.1	2.9	13.7	3.2	4.9	2.3	17.6	2.0	2.7	1.7	3.4
	3	4.5	11.9	3.0	10.1	2.3	8.3	3.3	6.5	3.5	1.0	1.0	0.8
	6	6.5	5.4	5.8	5.2	3.7	5.7	2.5	15.0	2.7	2.6	1.0	2.1
Mean		5.1	11.1	3.9	9.7	3.1	6.3	2.7	13.0	2.7	2.1	1.2	2.1
II	1	6.7	11.7	7.4	14.7	3.6	3.6	3.7	6.0	2.5	2.9	1.2	3.5
	3	1.6	8.8	1.4	6.3	2.6	5.6	3.1	11.5	2.1	2.8	0.9	2.9
	6	2.7	9.0	1.6	9.6	3.0	6.3	2.8	15.8	2.6	2.3	0.9	2.9
Mean		3.7	9.8	3.5	10.2	3.1	5.2	3.2	11.1	2.4	2.7	1.0	3.1
III	1	2.2	7.3	3.2	16.2	3.3	3.9	3.5	8.3	2.1	2.7	0.9	3.1
	3	1.8	6.9	2.6	9.7	2.9	5.7	2.1	13.8	2.3	0.7	0.4	0.9
	6	3.7	6.8	5.3	7.6	3.6	2.9	3.6	13.9	-	-	-	-
Mean		2.6	7.0	3.7	11.2	3.2	4.2	3.1	12.0	2.2	1.7	0.7	2.0
IV	1	2.0	6.7	1.6	4.5	2.7	3.3	2.4	7.5	1.6	6.5	0.7	7.3
	3	3.7	7.6	4.4	9.0	1.5	5.6	1.8	13.3	-	-	-	-
	6	2.0	3.6	0.7	3.8	1.2	1.9	0.2	10.3	-	-	-	-
Mean		2.6	6.0	2.2	5.8	1.8	3.6	1.5	10.4	-	-	-	-
Grand Mean		4.5	8.5	3.3	9.2	2.8	4.8	2.6	11.6	2.4	2.7	1.0	3.0
0 Day		2.5	9.8	0.8	5.7	2.0	7.3	2.8	9.8	3.5	3.2	1.7	3.7

Table 34. EFFECT OF ANAEROBICALLY DIGESTED SEWAGE AND SOIL MOISTURE STATUS ON YIELD OF KENTUCKY 31 FESCUE. DATA EXPRESSED AS THE MEAN OF DRY WEIGHT (g) FOR THE 1, 3, & 6 MONTHS INCUBATION

Soil	Exp. No.	Field Capacity			Saturated		
		0	90T	224T	0	90T	224T
Ottokee Sand	I	0.049	0.237	0.174	0.125	1.206	1.065
	II	0.068	0.217	0.187	0.116	1.127	1.152
	III	0.069	0.171	0.251	0.207	1.439	2.152
	IV	0.067	0.193	0.182	0.246	1.417	1.439
	Mean	0.063	0.205	0.199	0.174	1.297	1.452
Celina Silt Loam	I	0.154	0.462	0.432	0.091	0.628	1.003
	II	0.128	0.383	0.391	0.087	0.468	1.027
	III	0.225	0.746	0.709	0.216	0.820	2.491
	IV	0.216	0.421	0.382	0.224	0.799	2.212
	Mean	0.181	0.503	0.479	0.154	0.679	1.683
Paulding Clay	I	0.200	0.498	0.268	0.895	1.656	1.862
	II	0.237	0.578	0.242	0.776	2.003	2.358
	III	0.271[a]	0.588[a]	0.224[a]	1.026[a]	1.381[a]	1.554[a]
	IV	0.055[a]	0.089[a]	0.040[a]	0.357[a]	2.333[a]	2.613[a]
	Mean	0.191	0.438	0.194	0.764	1.843	2.097

[a] Data for Paulding Clay is incomplete since no data was available for Exp. III, 6 mo. and Exp IV, 3 and 6 mo.

Table 35. NITROGEN AND SULFUR CONTENT OF KENTUCKY 31 FESCUE GROWN IN SLUDGE AMENDED SOILS.[a]

Soil Treatment	Ottokee Sand		Celina Silt Loam		Paulding Clay	
	N%	S%	N%	S%	N%	S%
Control-FC[b]	-	-	1.91	-	1.57	-
90 Ton-FC	2.70	0.34	3.39	0.29	3.24	-
224 Ton-FC	3.20	0.57	3.41	0.34	3.52	-
Control-Sat.[c]	1.30	0.10	1.17	0.28	1.00	-
90 Ton-Sat.	1.69	0.41	1.39	0.64	2.03	0.29
224 Ton-Sat.	2.51	0.52	2.46	0.46	2.67	0.34

[a] Data shown are the means for a particular soil sludge-soil moisture treatment over all incubation periods and experiments.

[b] FC = Field capacity

[c] Sat. = Saturated

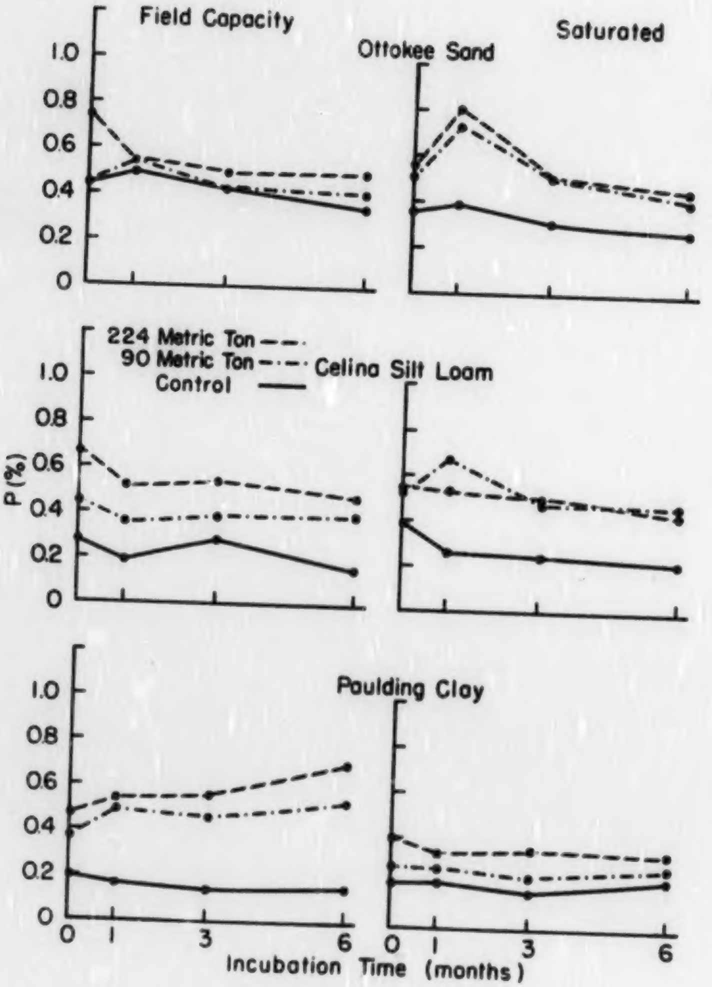

Figure 44. Phosphorus content of Kentucky 31 Fescue grown in sludge amended soils.

in the Ottokee sand at field capacity. Here the obviously poor growth may have altered the normal plant physiological processes and affected P uptake.

Potassium uptake by Kentucky 31 Fescue was unaffected by sludge additions to soils (Figure 45) except for a small positive response in the Ottokee sand. Since anaerobically digested sewage sludge adds relatively little K to soils this overall low response was expected. The Ottokee sand was so low in potassium (note the low K concentration in the control plants) that the addition of even low concentrations in the sludge resulted in increased plant uptake of K.

Plant content of Mg and Ca were largely unaffected by sewage sludge amendments (Figure 46 and 47). This was true even though the concentration of Ca and Mg in the displaced soil solutions (Tables 26 and 27) was increased considerably by sludge additions. Perhaps the increased uptake of Na by the Fescue (Figure 48) was responsible for a decreased uptake of the divalent Ca and Mg ions.

Sodium uptake by Kentucky 31 Fescue was increased consistently in all soils and at all moisture contents (Figure 48). These data reflect the relatively large additions of Na with the sludge. The Na content of the displaced soil solution of the Ottokee sand and Celina silt loam increased about 6 - 8 fold because of the sludge amendment (Table 28). The increase in the soil solution of the Paulding clay was neglible but the activity of exchangeable sodium undoubtedly remained high enough to provide large quantities of Na for plant uptake.

The three nutrient elements Cu, Zn, and B showed similar uptake patterns from sludge amended soils. Decreased uptake of all three elements was associated with an increase in soil moisture saturation and reduced aeration (See Figures 49, 50, and 51). The greatest reduction in uptake was evident in the Paulding clay, while Celina silt loam gave an intermediate response. In the coarse textured Ottokee sand, water saturation had no effect on the uptake of Zn, Cu and B. Reductions in Zn, Cu and B in corn grown on fine textured soils were previously observed by Lal and Taylor (1970) in lysimeters with shallow water tables or those flooded intermittently. These authors attributed this reduced uptake to decreased ion solubility caused by their coprecipitation with soluble Al and Fe in soils under reducing conditions. In this study other explanations seem more plausible. Since there is a direct relationship between microbial activity as determined by CO_2 evolution and the uptake of Zn, Cu and B, we can speculate that the response is due to mineralization of these elements bound to sludge organics or by the greater production of low molecular weight organic ligands which would influence ion, solubility and availability. Alternatively, an indirect effect of microbial activity, namely the reduction of pH by nitrification and the increased solubility of Zn, Cu and possibly B, may also be of significance. Unfortunately there does not seem to be a relationship between the concentration of Zn and Cu in the displaced soil solution (Tables 29 and 30) and plant uptake of these elements.

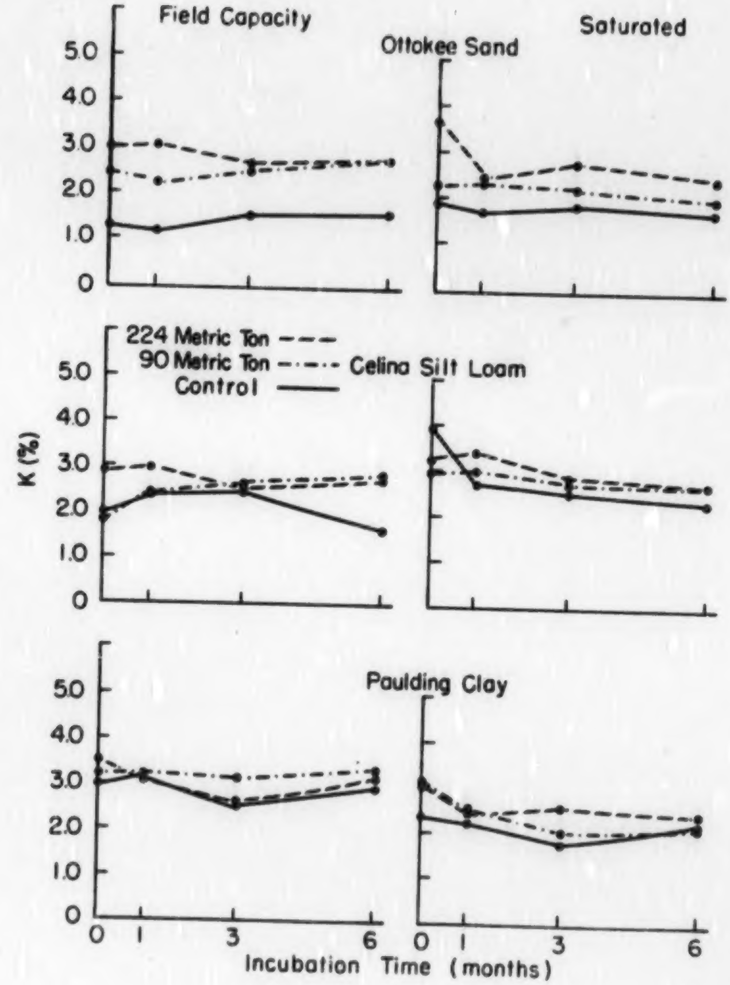

Figure 45. Potassium content of Kentucky 31 Fescue grown in sludge amended soils.

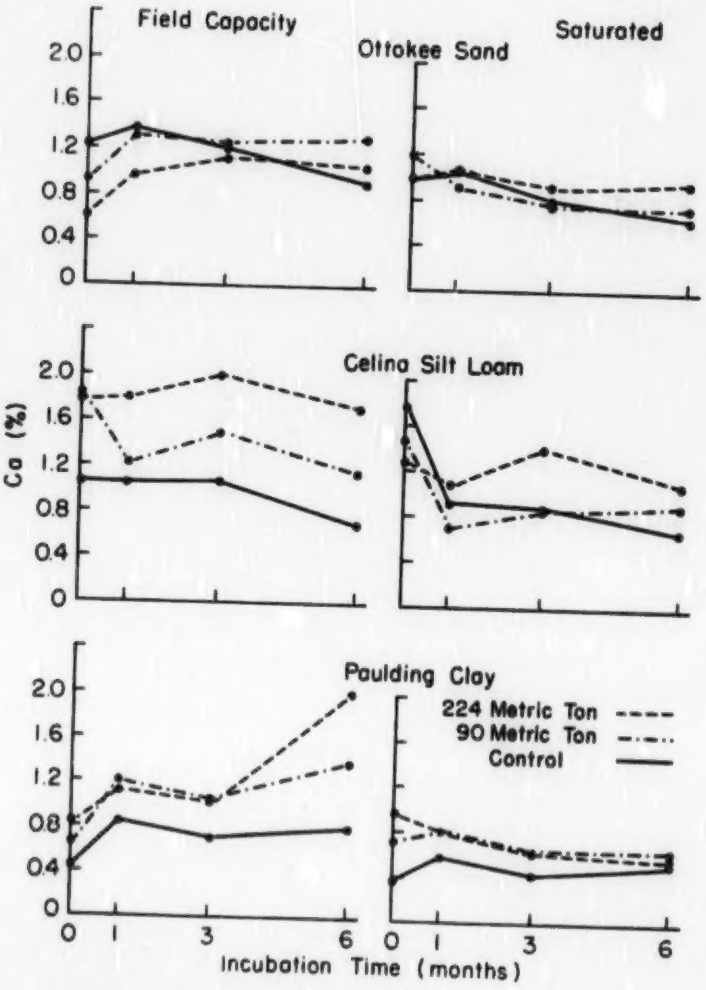

Figure 46. Calcium in content of Kentucky 31 Fescue grown in sludge amended soils.

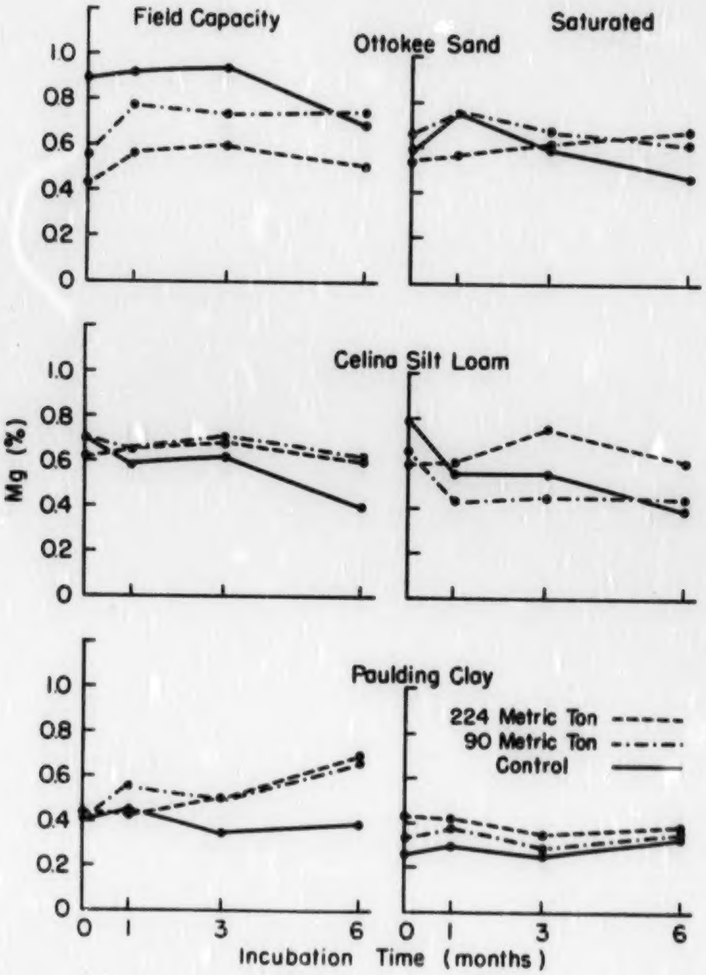

Figure 47. Magnesium content of Kentucky 31 Fescue grown in sludge amended soils.

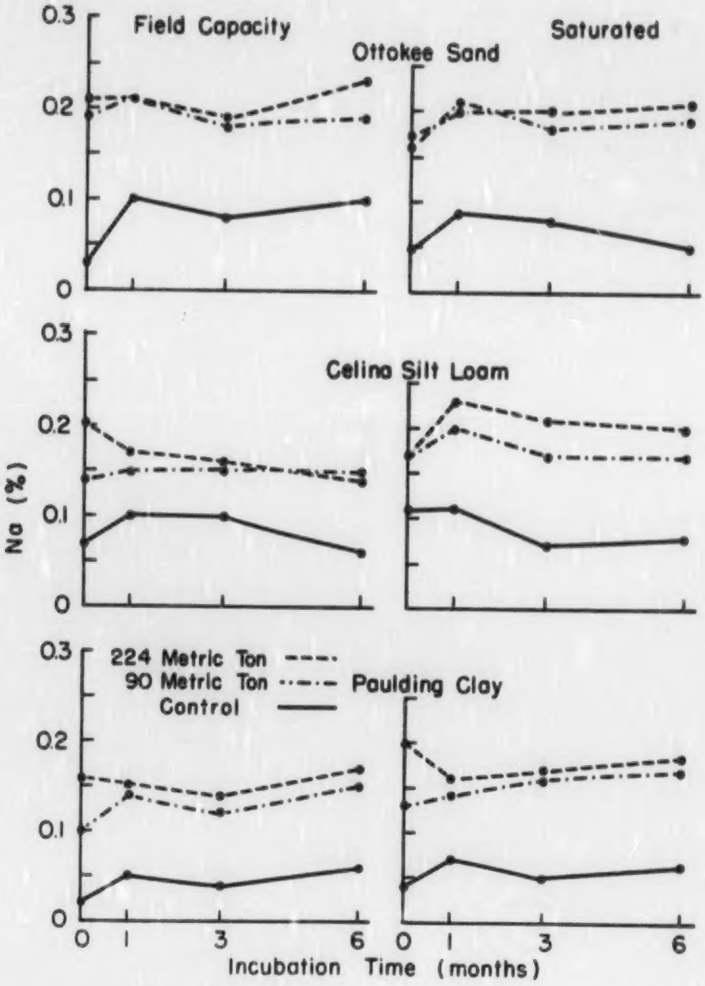

Figure 48. Sodium content of Kentucky 31 Fescue grown in sludge amended soils.

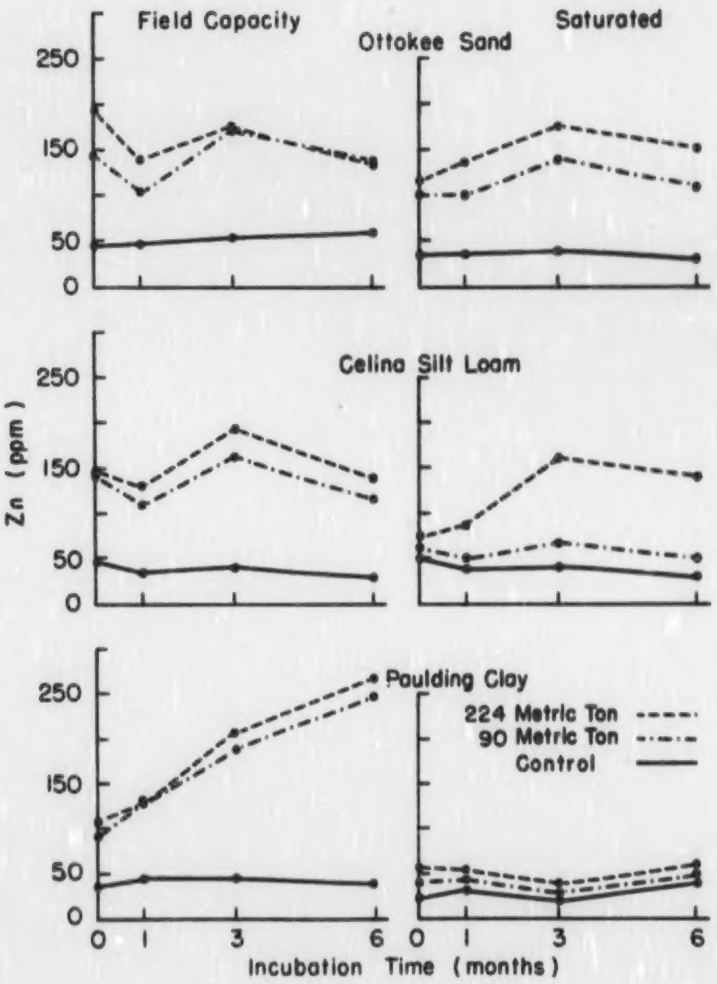

Figure 49. Zinc content of Kentucky 31 Fescue grown in sludge amended soils.

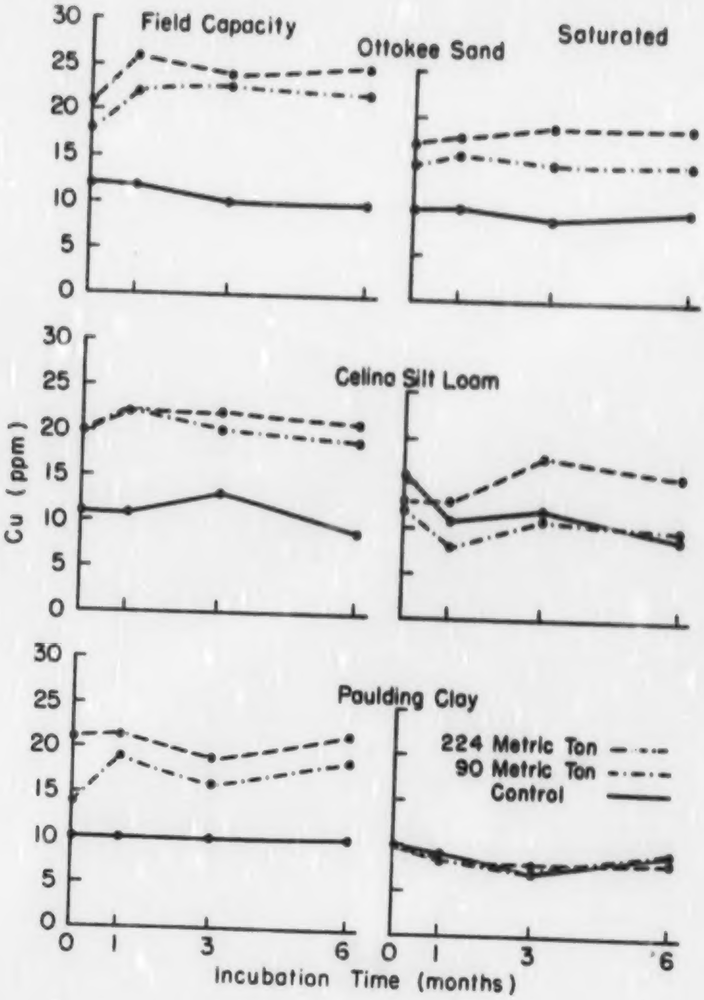

Figure 50. Copper content of Kentucky 31 Fescue grown in sludge amended soils.

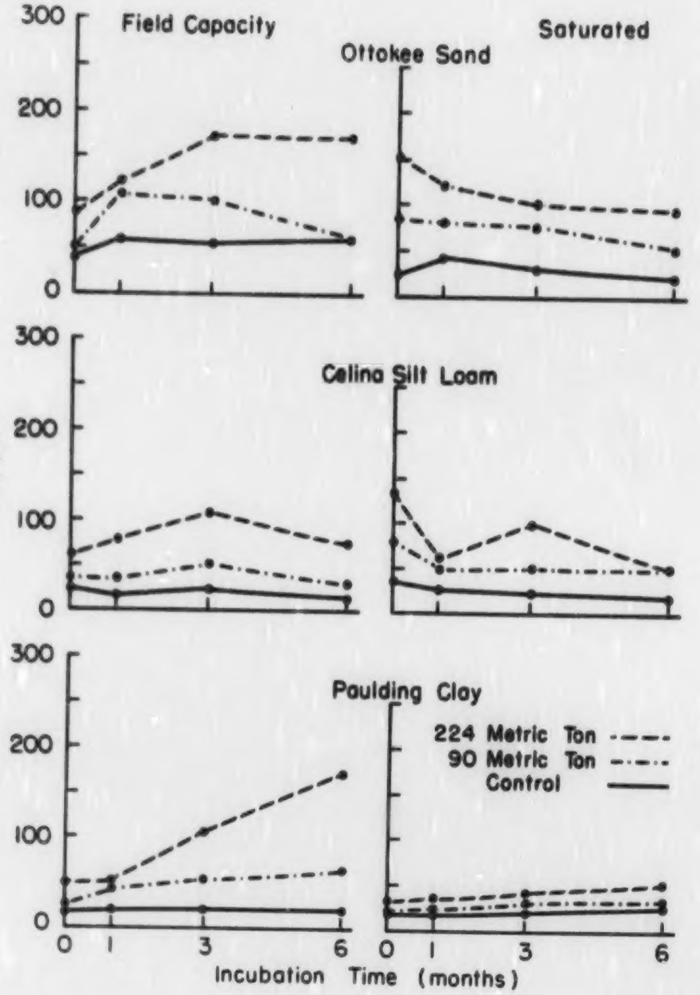

Figure 51. Boron content of Kentucky 31 Fescue grown in sludge amended soils.

Manganese availability and uptake by Kentucky 31 Fescue is shown in Figure 52. Although there is a trend for the plant uptake of Mn to follow that of Zn, Cu, and B discussed previously, there are enough inconsistencies to make interpretation difficult. Also disconcerting is a lack of correspondence between the concentration of Mn in the displaced soil solution (Table 31) and Mn uptake. This may be attributable to the ease of oxidation-reduction reactions of Mn in soil influenced by microbial activity, organic matter content, and water saturation. Data for plant analysis of Al, Fe, Sr, Ba, and Mo were also obtained but are not included because there were no differences between plants grown in sludge amended or control soils.

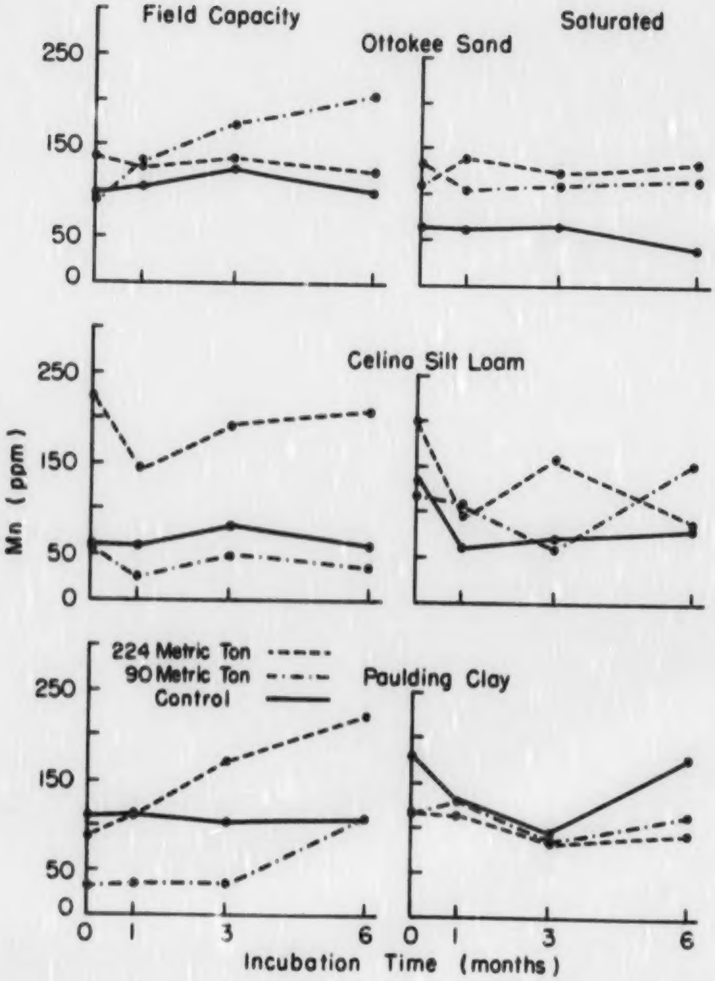

Figure 52. Manganese content of Kentucky 31 Fescue grown in sludge amended soils.

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Appendix A

Sludge - Soil Extract Agar

1.0g Glucose

0.5g K_2HPO_4

750 ml Sludge Extract*

100 ml Soil Extract**

150 ml Tap Water

* Preparation of Sludge Extract - One liter of anaerobically digested sewage sludge was autoclaved at 121°C for 30 minutes. The suspension was filtered through Whatman #1 filter paper using Celite filter aid.

** Preparation of Soil Extract - One liter of tap water was added to 1000g of a fertile soil and autoclaved at 121°C for 20 minutes. Colloids were flocculated with 0.5g $CaCO_3$ and the suspension filtered through Whatman #1 filter paper.